ARCHIVES OF PATHOLOGY

VOLUME 33 MARCH 1942 NUMBER 3

OBSERVATIONS ON DRY FILMS OF CULTURES OF LYMPHOID TISSUE

LAWRENCE BERMAN, M.D.

DETROIT

The important role of the lymphocyte and the functional alterations of hematogenous and histogenous elements in inflammation were emphasized by Maximow in a series of studies of reacting tissues, beginning in 1902, and tissue cultures, beginning in 1916. His observations and those of workers who have confirmed and extended them [1] reveal that lymphocytes from tissues, blood and lymph under suitable conditions will transform into ameboid wandering cells, macrophages,[2] epithelioid cells, giant cells,[3] fibroblasts [4] and granulocytes.[5]

The bulk of evidence based on studies of inflammation in animals and tissue cultures indicates a dual origin of polyblasts, mononuclear macrophages, giant cells and endothelial cells, first, from histogenous elements, i. e., clasmatocytes and reticuloendothelial cells, and, second, from hematogenous cells, i. e., lymphocytes and monocytes. This information has been obtained from examination of exudates, sectioned and stained tissues, subcutaneous tissue spreads, unstained and supravitally stained tissue cultures and fixed sectioned or whole mounts of tissue cultures. It is probable that fixed sectioned material, as well as unstained and

---

The work was performed in the Tissue Culture Laboratory of the Department of Physiology, University of Minnesota, Minneapolis.

From the Departments of Anatomy and Pathology, University of Minnesota, and the Department of Pathology, Wayne University College of Medicine.

1. For a detailed bibliography the reader is referred to the discussion of tissue cultures in Downey, H.: Handbook of Hematology, New York, Paul B. Hoeber, Inc., 1938, section XX.

2. (*a*) Maximow, A.: Proc. Soc. Exper. Biol. & Med. **24**:570, 1927. (*b*) Bloom, W.: ibid. **24**:567, 1927. (*c*) Latta, J. S., and Johnson, H. N.: Arch. f. exper. Zellforsch. **16**:221, 1934.

3. (*a*) Maximow, A.: J. Infect. Dis. **34**:549, 1924; (*b*) **37**:418, 1925. (*c*) Lewis, W. H., and Webster, L. T.: J. Exper. Med. **34**:397, 1921.

4. Maximow.[2a] Lewis and Webster.[3c]

5. (*a*) Bloom, W.: Tissue Cultures of Blood and Blood-Forming Tissues, in Downey, H.: Handbook of Hematology, New York, Paul B. Hoeber, Inc., 1938, vol. 2, p. 1514. (*b*) Latta and Johnson.[2c]

supravitally stained living material, showing functional and morphologic changes of lymphocytes and macrophages has not been more impressive because of the difficulty of distinguishing various cell types in sections as compared with similar cells in dry films or imprints treated with Wright's or May-Grünwald-Giemsa stains.

The need for a dry film technic for morphologic studies of inflammation was partly answered by Kolauch,[6] who devised a method by which subcutaneous tissue spreads of inflammatory lesions were rapidly dried in air and thereafter treated as films of blood. The reported observations which dealt in detail with the transformations of clasmatocytes and lymphocytes into macrophages were in perfect agreement with the conclusions repeatedly made by Maximow and regarded as proved beyond doubt by Bloom.[7] Kolauch's experiment was terminated at the seventy-sixth hour stage, at which time the histogenous and hematogenous macrophages had become morphologically identical. For some studies of inflammation and cellular transformations it is desirable to isolate the tissues in order to simplify, control or prolong the experimental conditions. Therefore, a method has been devised for obtaining dry films from tissue cultures of blood and hemopoietic tissues. In a discussion of the application of tissue culture technics to hematologic problems, Bloom [8] stated, "Unfortunately, satisfactory methods for the preparation of dry smears of cultures have not been developed." The simple "blot" technic described in the following paragraphs seems to remove that deficiency. The observations reported herein are obtained from several series of rabbit mesenteric lymph nodes.

METHOD

The material used to develop the method consisted of several series of cultures of mesenteric lymph nodes of adult rabbits. The tissue was grown in homoplastic heparinized plasma to which serum chick embryo extract was added. The double cover slip method was used, as modified and previously described by King.[9] Some of the older cultures were washed with Tyrode's solution and refed with plasma and serum extract. Most of the cultures were planted by Mrs. Marian Kaplan for use as controls of another experiment and as such they were quite uniform in size, rate of migration and development.

The object of this method is to obtain a thin layer of migrating cells which may be rapidly dried in air and subsequently treated as a film of blood and stained with the May-Grünwald-Giemsa combination. This is accomplished simply by removing most of the fluid from the culture by blotting. In that process a great deal of fibrin on the upper surface of the clot is also removed. On the cover slip there remains a thin moist film of fibrin and cells. After one has dried the

6. Kolauch, F.: Am. J. Path. **15**:413, 1939.

7. Bloom, W., in Maximow, A., and Bloom, W.: A Textbook of Histology, ed. 3, Philadelphia, W. B. Saunders Company, 1938, p. 107.

8. Bloom,[8a] p. 1474.

9. King, J. T.: Arch. f. exper. Zellforsch. **9**:341, 1930; **10**:467, 1931; **20**:208, 1937.

film rapidly by whipping it in the air, it is ready for staining. The steps in the process are as follows:

1. Prepare a mount by placing a drop of balsam or mounting solution on a clean microscope slide.
2. Remove the cover slip carrying the culture.
3. Quickly place the cover slip on absorbent filter paper, glass side down. Within a few seconds the small amount of moisture remaining on the bottom of the cover slip will have been removed.
4. Mount the cover slip on the microscope slide previously prepared with a drop of balsam. This will provide a convenient means of handling the specimen for subsequent maneuvers.
5. Immediately after having mounted the specimen, cover the preparation with a sheet of filter paper. Allow fluid to be absorbed from the clot. No pressure should be applied, and there must be no slipping action of the paper over the surface of the clot. This can be prevented by anchoring both the end of the slide and the filter paper under the thumb. The filter paper is allowed to drop gently on the surface of the clot, and almost at once it is peeled off. Then shift the filter paper so that a dry area overlies the clot, and repeat the operation. Usually six or seven "blots" will be sufficient. The operator must acquire skill in judging the point at which enough of the clot has been removed. When too much has been blotted off, the cells are injured, and when too little has been removed, the film is too thick for proper staining.
6. Dry the moist film as rapidly as possible in air. This will take only ten to twenty seconds if the film is of correct thickness.
7. Stain with the May-Grünwald-Giemsa combination in the same manner as imprints or smears of bone marrow are treated. It has been found satisfactory to apply the May-Grünwald stain for one minute, then the Giemsa solution for seven minutes, and differentiate in distilled water. The Giemsa stain is diluted in buffer (p_H 6.4) so that each cubic centimeter of diluted Giemsa solution contains 2 drops of stain.

It should be pointed out that very careful section of filter paper for blotting is necessary. For this purpose the paper should be lintless, fine grained, smooth and fairly hard. A number of absorbent surfaces were tried out, but it was found that the smooth, lintless papers used for filtering intravenous solutions are most satisfactory. During the process of blotting, the original fragment of tissue is removed, as it sticks to the paper. The specimens are thus usually limited to the cells in the migration zone. This constitutes the most serious disadvantage of the method. On the other hand, the character of the migration zone is often faithfully preserved (fig. 2 *A, B* and *C*). Although the migration ring may be incomplete or variable in thickness, there are usually areas in which the film is very thin; in these parts the cells are sharply stained against an almost clear background. Occasionally cells in the original explant area remain adherent to the cover slip and afford good opportunities for study. The rapid removal of excess material and the rapid drying of the preparation appear to arrest the cells in the positions and shapes they had during their life in vitro. Ameboid forms are visible, and it has been possible to recognize the forms of cells described in unstained living cultures with the advantage of having the nuclear and cytoplasmic details filled in.

OBSERVATIONS

The behavior of explanted lymph node has been described by Maximow and others. It suffices to point out that during the first twenty-four hours of incubation the explants in our series were surrounded by a zone of migrating ameboid cells, most of which were lymphocytes of various sizes. Wandering reticular cells soon appeared in the migration zone. In the meantime the surviving lymphocytes exhibited hypertrophy. At the end of forty-eight to sixty hours the polyblasts [10] of reticular and lymphocytic origin came to resemble each other very closely. There was enough cellular debris to afford many of the cells the occasion to function as macrophages.

As seen in dry films of tissue cultures, the change from lymphocyte to polyblast is characterized by alterations of the cytoplasm, the nucleus and the apparent type of motion. Within four hours many of the lymphocytes degenerate. In the migration zone practically all the cells are lymphocytes of different types or sizes (fig. 1 *A*). At the end of four hours vacuolation of the cytoplasm has occurred in the majority of the migrating cells. The vacuolation is more abundant near the nucleus; there is usually a more homogeneous rim of basophilic cytoplasm at the periphery. As a rule, the smaller lymphocytes tend to remain round. Scattered about there are occasional very large cells, which are quite conspicuous because of their deeply basophilic cytoplasm, which contains occasional large vacuoles (fig. 1 *A*). These have round, sharply defined nuclei, which include one or more nucleoli, obscured by the overlying darkly staining irregular but small chromatin particles, which are arranged in small aggregations. These are the large lymphocytes of reticular origin. The nuclei of the large cells are relatively immature as judged by standards of dry film technics. While the basophilia thus appears to be an attribute of these relatively large, immature cells, as advocated by Wiseman,[11] it is not confined to cells of that type. Many ameboid lymphocytes, including those with typically pachychromatic nuclei, have deeply basophilic cytoplasm, especially in the nonvacuolated parts. The nuclear chromatin of these active lymphocytes shows greater dispersion and smaller particles. There are a few cells with large and slightly irregular lymphocytic nuclei and abundant cytoplasm in ameboid contours. Occasional mitoses are seen.

In one specimen, at the end of eighteen and a half hours the migration zone contained lymphocytes of various sizes. There was general hypertrophy of all. The larger cells were much larger than any of the cells of the four hour stage, even the cells with small lymphocytic nuclei had relatively abundant cytoplasm. Vacuolation of the cytoplasm was one

10. The term "polyblast" as used here indicates a wandering cell other than a typical lymphocyte.

11. Wiseman, B. K.: J. Exper. Med. **54**:271, 1931.

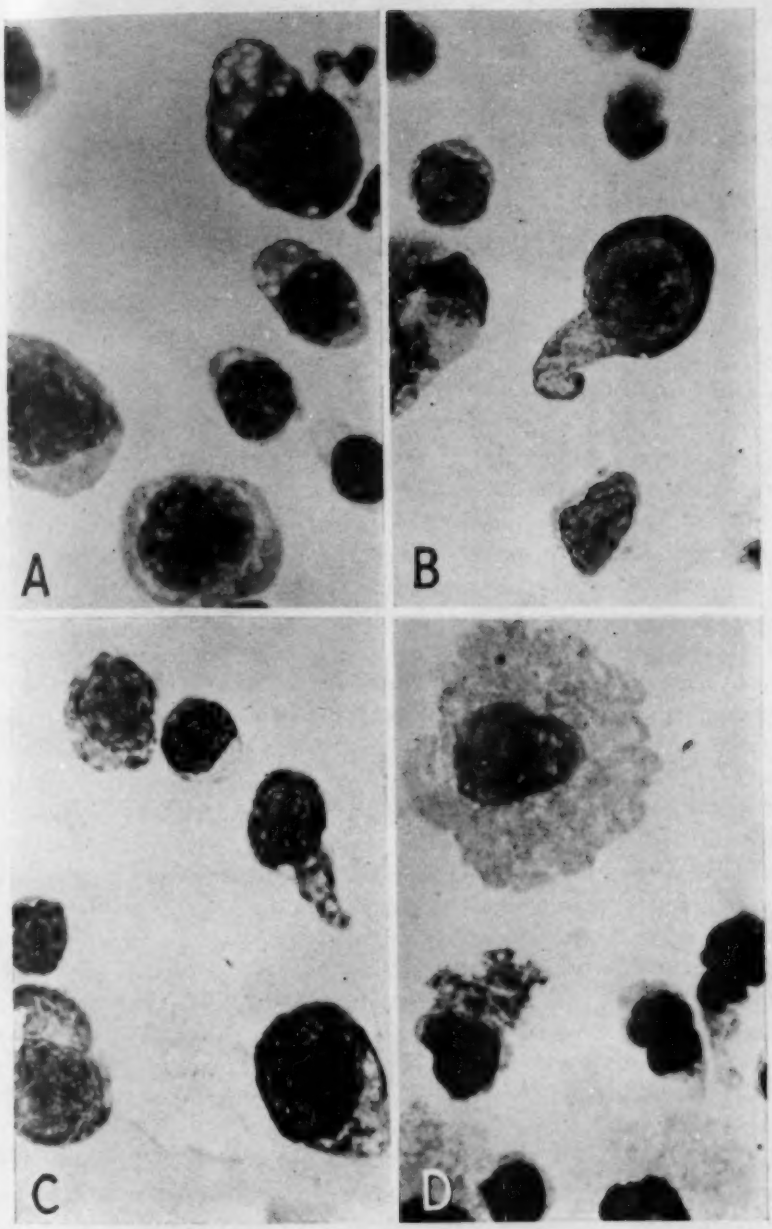


Fig. 1.—*A*, lymphocytes in the migration zone of an early culture. The cell in the upper right hand corner has deeply basophilic cytoplasm. The vacuoles tend to occupy one hemisphere of the cell. The parachromatin network of the nuclei has become more distinct. × 1,100. *B*, hand mirror form of large migrating lymphocyte in the migration zone of an eighteen and one-half hour culture. × 1,100. *C*, hand mirror form of small lymphocyte in an eighteen and one-half hour culture. The cell in the lower left hand corner is a lymphocyte in which the nucleus is beginning to lose its compact structure. × 1,000. *D*, hand mirror forms in small lymphocytes. The large cell is a polyblast of lymphocytic origin. × 1,100.

sided, as in other specimens of this age. There were occasional hand mirror forms of migrating lymphocytes, which must be similar if not identical to the forms described by W. H. Lewis [12] for migrating lymphocytes and by Rich, Wintrobe and M. R. Lewis [13] for lymphoblasts (fig. 1 *A*, *B* and *C*). The blunt rounded and wider anterior portion of the migrating lymphocyte is the homogeneous portion and nucleus, and the tail is the vacuolated part.

At the end of twenty-four hours there are many ameboid lymphocytes. In several cultures of this age there was opportunity to observe the changes which had occurred in the area of the explant, as a thin layer of cells remained adherent to the cover slip. In these slides it is possible to recognize free reticular cells, some of which contain greenish material in their cytoplasm. These cells are easily recognized as being nonlymphocytic in the dry preparations. They have wrinkled, often folded nuclei, sometimes with one or more blue-staining nucleoli. The nuclear pattern is delicately stippled, with only little tendency to clumping of chromatin. The cytoplasm is filled with fine vacuoles or pale-staining areas, giving it a foamy or reticulated appearance.

In cultures of the thirty-one hour stage there are free reticular polyblasts in the migration zone. The majority of the lymphocytes have undergone further change of nuclear structure toward more distinct separation of chromatin and parachromatin and smaller size of chromatin particles. At this stage it becomes possible to separate out different groups of cells on the basis of the behavior and appearance of the cytoplasm. The first group have round, ameboid or hand mirror form, with one side of the cell presenting a vacuolated structure and the opposite, anterior end presenting homogeneous cytoplasm. This group of cells includes all those previously recognized as lymphocytes, including small lymphocytes with typical pachychromatic nuclei, medium-sized lymphocytes with nuclei of looser structure and the large cells with nuclei containing evenly dispersed irregular chromatin fragments, and often including distinct nucleoli. A second group are those cells with abundant very finely vacuolated or reticulated foamy cytoplasm, oriented about the nucleus in a manner which suggests no polarity with regard to direction. The contour of these cells is often lobulated or wavy because of the great number of small round pseudopodia which seem to extend in all directions.

In addition, some cells resembling lymphocytes because of the clumping of chromatin in the nucleus differ from the reticular cells only slightly. These appear to be lymphocytes which are in the stage of transforming into polyblasts of indifferent origin, i. e., polyblasts whose ancestry from reticular cell or lymphocyte cannot be differ-

12. Lewis, W. H.: Bull. Johns Hopkins Hosp. **49**:29, 1931.

13. Rich, A. R.; Wintrobe, M. M., and Lewis, M. R.: Bull. Johns Hopkins Hosp. **65**:291, 1939.

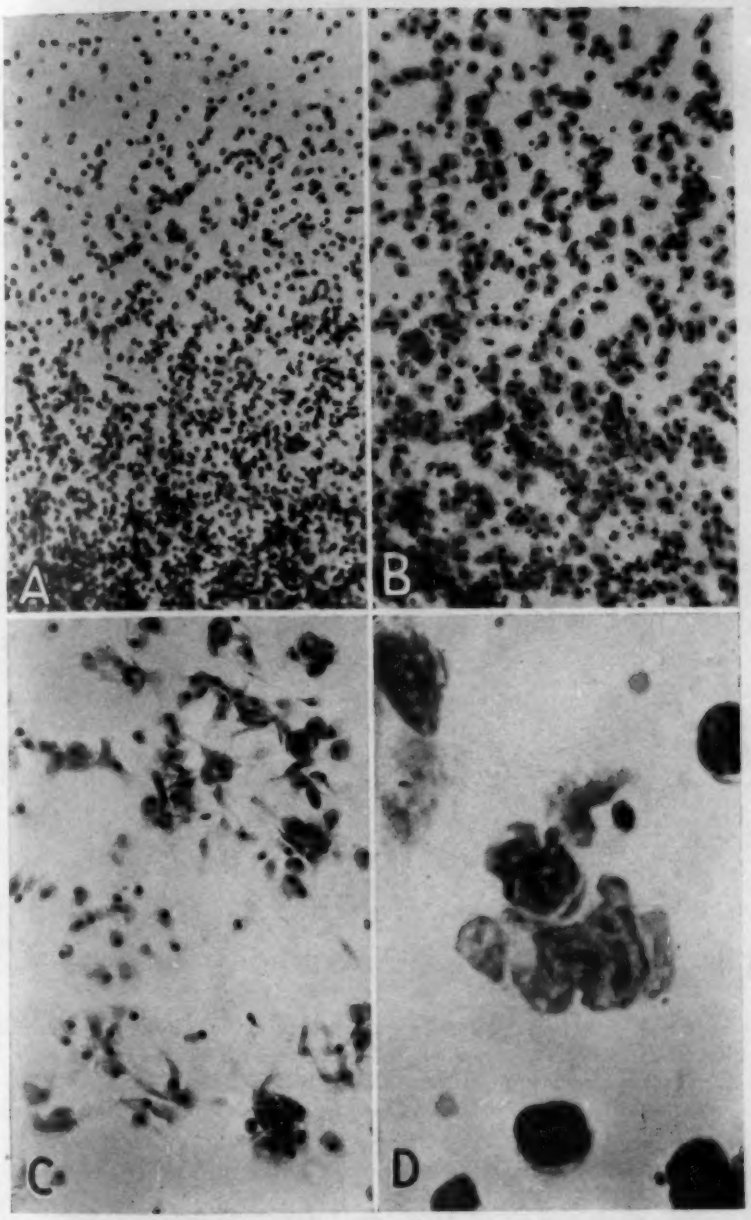


Fig. 2.—*A*, the migration zone of a thirty-one hour culture. The majority of the cells are lymphocytes, but there are scattered free reticular wandering cells. × 100. *B*, the migration zone of a ninety-two hour culture. Most of the cells are polyblasts of indifferent origin. There are many mitotic figures. × 100. *C*, the migration zone of a four hundred and thirty-two hour culture. Some of the cells have fused. There are numerous elongated or spindle forms with expanded pseudopodia. The nuclei are round or slightly oval. × 100. *D*, a lymphoid polyblast in contact with a small lymphocyte. The nucleus of the large cell has lost its compact structure. × 1,100.

entiated with certainty (figs. 1 *C* and *D*, 2 *D* and 3 *A*). It is suggested that the type of motility as indicated by shape and position of pseudopodia might be taken to indicate the functional stage of the cell, not always its origin.

At fifty-one hours the migration zone contains large numbers of polyblasts, many of which are definitely macrophages containing debris. Some of these cells contain greenish pigment; others appear pink because of the presence of minute pink dust or granulation. There has been a further marked increase in the number and proportion of large polyblasts, now mainly of indifferent origin (fig. 3 *A*). There are still many

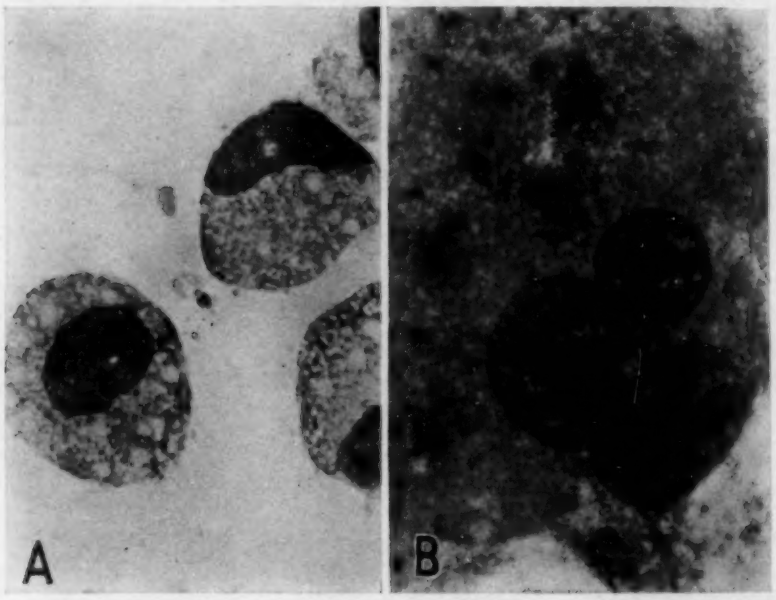


Fig. 3.—*A*, polyblasts of indifferent origin, from a fifty-one hour culture. × 1,100. *B*, a multinucleated cell from a one hundred and twenty hour culture. The nuclei have smooth, distinct membranes. The nucleoli are large and distinct. This is the third type of nucleus seen in cultures over fifty hours of age. × 1,100.

small and medium-sized lymphocytes in the migration zone. These cells do not appear to have changed much from ordinary lymphocytes except for some evidence of ameboid forms. The nuclei of the macrophages and large polyblasts are oval and often slightly irregular in contour. Nucleoli are visible in some of the cells. Among the polyblasts there are now several types of nuclei. One type has a fairly uniform size and distribution of chromatin particles. These appear to be the nuclei of large polyblasts of reticular origin. In another type of polyblast, the nucleus shows definite clumping of chromatin. These resemble the nuclei

of lymphocytes more closely. A third type includes round or slightly oval nuclei with sharply outlined and very smooth nuclear membranes enclosing evenly distributed chromatin granules, which are coarser than those seen in the reticular cells. These nuclei are often but not always slightly smaller and possess single large blue-staining nucleoli. Occasionally more than one nucleus of this type is present in a single cell (fig. 3 *B*). These cells appear to have arisen from polyblasts. The chief changes are rounding up of the nucleus, rearrangement of nuelear chromatin and development of a prominent nucleolus.

In one of the eighty hour slides the polyblasts have become darker and rounder. They nearly all have the third type of nucleus described for the fifty-one hour stage. The nucleus has become smaller, and the entire cell has become more compact. This appears to be due chiefly to a decrease in the number of vacuoles and a condensation which gives the cytoplasm a more solid and homogeneous appearance. The remaining lymphocytes are very small or pyknotic. In a film of the ninety-two hour stage practically all the cells in the migration area are rounded polyblasts with basophilic, finely vacuolated cytoplasm (fig. 2 *B*). Many of these rounded polyblasts seem to have arisen by mitosis, as the field contains a great many mitotic figures. The nuclei of the daughter cells are fairly compact.

After one hundred and sixteen hours the appearance of the cultures has changed considerably. Only a few small or pyknotic lymphocytes can be recognized. Some of the rounded polyblasts have fused into multinucleated masses. A few cells have sprouted clear fan-shaped pseudopodia by which they appear to be attached to the glass. The majority of the cells are round or oval. Their cytoplasm contains few vacuoles, and the nuclei are identical to the third type described in the fifty-one hour culture. One cell contains a large nuclear mass which is undergoing multiple division, showing two well formed smaller daughter nuclei in the process of budding off, each with its own nucleolus, and a third nuclear mass containing two nucleoli.

Subsequent changes in the cultures progressed at a very slow rate. Observations were made on groups of cultures at approximately one hundred and twenty, one hundred and forty, two hundred, three hundred and thirty and four hundred and thirty hour stages. The oldest culture in the series was dried and stained after four hundred and thirty-two hours. The essential change after the one hundred and sixteen hour stage was in the character and orientation of the cytoplasm of the polyblasts and in the appearance of giant cells in the cultures. As the cytoplasm of the large rounded or oval polyblasts became more homogeneous, fan-shaped pseudopodia projected out in various directions. This resulted in the formation of many bizarre forms, including spindle, triangular

and fibroblast-like shapes (fig. 2 *C*). The nuclei remained round, with distinct nucleoli and fairly evenly dispersed chromatin. The fibroblast-like cells were compared with cultures of fibroblasts stained in dry films, and although the nuclear pattern was morphologically identical in the two kinds of cells, the nuclei of the cells in the fibroblast cultures were elongated or oval instead of round as in the polyblast cultures. Furthermore, the processes of fibroblasts in the dry films were clearly defined and often sharply pointed. The stretched-out triangular and spindle forms of polyblasts in the four hundred and thirty-two hour culture usually had fan-shaped extremities which became so attenuated that a definite edge could not be discerned.

Most of the cells in the older cultures occurred in small groups and masses. Morphologically such groups of cells resembled quite closely those seen in imprints of tuberculous hyperplasic human lymph nodes. It was felt that many of the cells in the one hundred and twenty to four hundred and thirty-two hour cultures bore a close resemblance to epithelioid cells. It also seemed clear that giant cells, which were found in increasing frequency as the age of cultures increased, were formed as the result of either fusion of polyblasts or division of nuclei, as transition forms for both types of giant cells could be found.

SUMMARY

A method is described for obtaining dry films from tissue cultures of blood and hemopoietic tissue.

Observations made on a series of cultures of adult rabbit lymph nodes by means of the blot technic reveal the cytologic details of a transformation of lymphocytes and reticular cells into polyblasts, and of polyblasts into macrophages, epithelioid cells and giant cells.

The characteristic forms of migrating lymphocytes described in living unstained cultures are preserved in the dry film.

Typical localized vacuolation seen in the lymphocytes of the dry films is replaced by diffuse vacuolation at the time when both the directional polarity of the cell and the lymphocytic character of the nucleus are lost. This accompanies a change in the type and the location of pseudopodia and represents the point of transition from a lymphocyte to a polyblast of indifferent origin.

The change from polyblast to epithelioid cell or fibroblast-like cell is associated with the appearance of a prominent nucleolus and a change in the nucleus, which assumes a more circular form and acquires a distinct smooth membrane.

Giant cell formation occurs both by fusion of cells and by multiple division of nuclei.

CHANGES IN OSSEOUS TISSUES OF YOUNG DOGS AFTER PROLONGED ADMINISTRATION OF ESTRADIOL BENZOATE

CHARLES J. SUTRO, M.D.
AND
LEO POMERANTZ, B.S.
NEW YORK

It has been demonstrated that when young mice of certain strains received repeated injections of estrogenic substances, they developed osteosclerosis.[1] Little is known, however, of the changes occurring in the osseous tissues of young dogs after prolonged administration of such estrogens.[2] In this paper we report a study of this question.

METHODS

Eight mongrel puppies were the subjects of experiment. Six of them, 3 males and 3 females, were from one litter, and the remaining 2, a male and a female, were from another. The average weight of the 6 dogs was about 2.9 Kg., while the remaining 2 weighed 1.4 and 1.6 Kg., respectively. Three of the 8 puppies, 2 males and 1 female, served as controls. The 5 experimental subjects received subcutaneous injections of estradiol benzoate in sesame oil in doses of 10,000 to 20,000 rat units per week for periods of five to seven and a half months [3] (table). The three control animals received subcutaneous injections of sesame oil alone each week for similar periods of time.

Experimental Data

Sex	Weight at Onset Kg.	Duration of Experiment, Mo.	Weight at Termination of Experiment, Kg.	Dose of Estrogen per Week, Rat Units	Total Amount of Estrogen, Rat Units
F	3.0	5	9.6	10,000 to 20,000	256,000
F	2.8	7½	8.6	10,000 to 20,000	465,000
F	1.6	5	6.2	10,000 to 20,000	396,000
M	1.4	5	6.2	10,000 to 20,000	296,000
M	2.8	7½	7.4	10,000 to 20,000	485,000

The diet consisted of meat, cod liver oil, soy beans, milk, Purina chow cubes and water.

From the Laboratory of the Hospital for Joint Diseases.

1. Zondek, B.: Folia clin. orient. **1**:1, 1937. Gardner, W. U., and Pfeiffer, C. A.: Proc. Soc. Exper. Biol. & Med. **37**:678, 1938; **38**:599, 1938. Sutro, C. J.: ibid. **44**:151, 1940.

2. Tausk, M., and de Fremery, P.: Acta brev. Neerland. **5**:19, 1935.

3. Estradiol benzoate in sesame oil was supplied by the Schering Corporation.

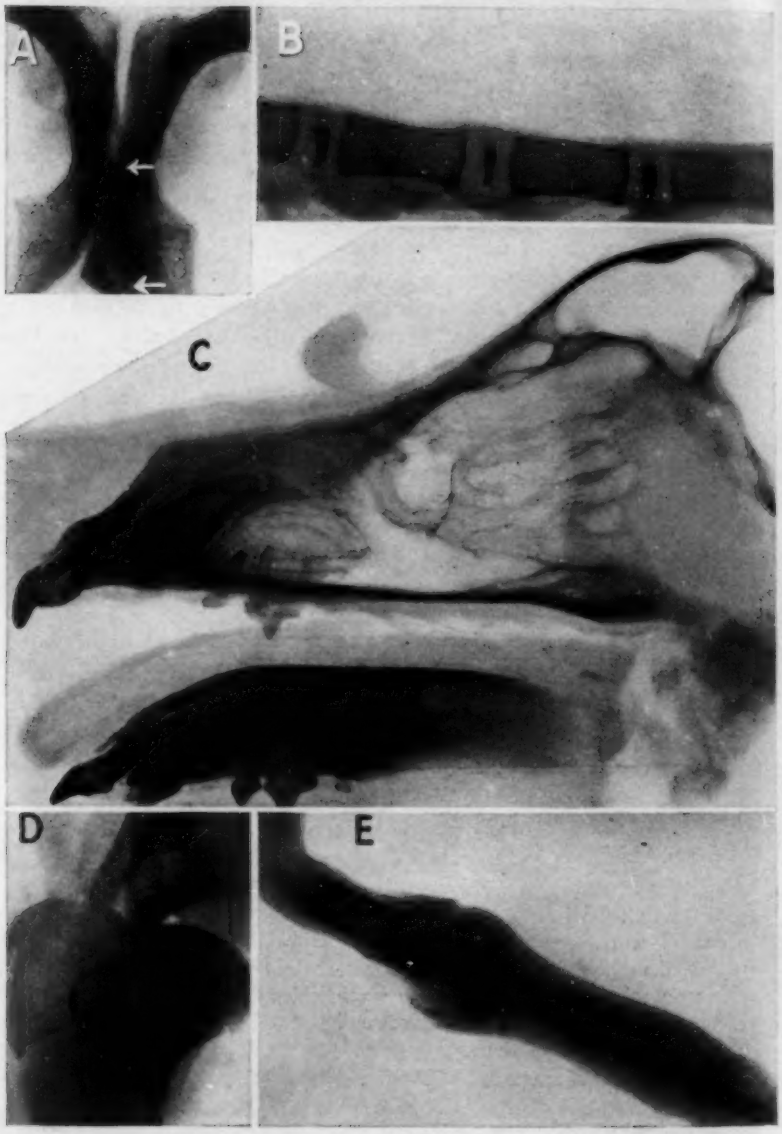


Fig. 1.—Roentgenograms of a normal male adult dog: In *A*, note the island of bone occupying the lower half of the pubic symphysis (arrows); in *B*, the height of the bodies of the sternum; in *C*, the length of the snout and the size of the frontal sinus (the lower jaw has been inadvertently turned on the film); in *D*, the open epiphysial plate region in the upper end of the humerus; in *E*, bone occupying almost the entire length of the penis.

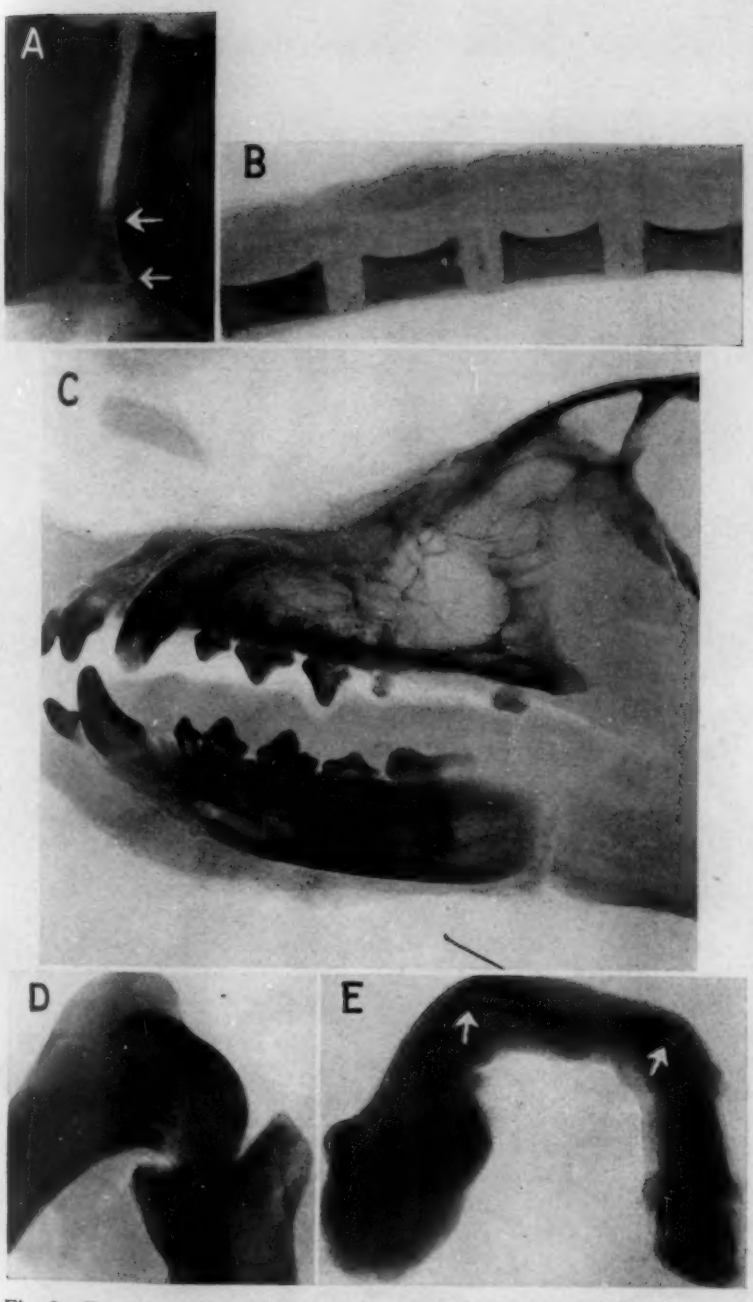


Fig. 2.—Roentgenograms of a male dog which had received 485,000 rat units of an estrogen in sesame oil subcutaneously for a period of seven and one-half months: In *A*, note island of bone occupying only a small portion of the pubic symphysis (arrow); in *B*, the short bodies of the sternum; in *C*, the short snout and small frontal sinus; in *D*, the closed epiphysial plate region in the upper end of the humerus; in *E*, the thin segment of bone occupying the midthird of the penis (arrows).

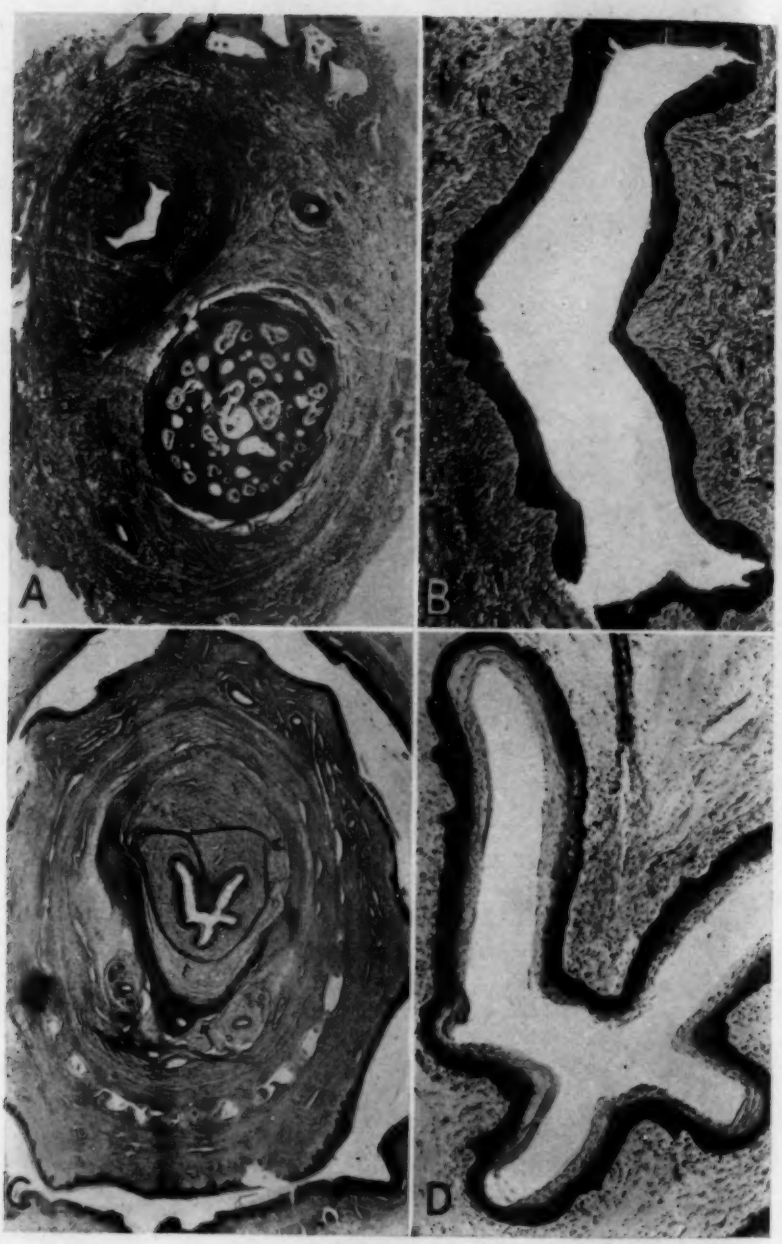


Figure 3

(See legend on opposite page)

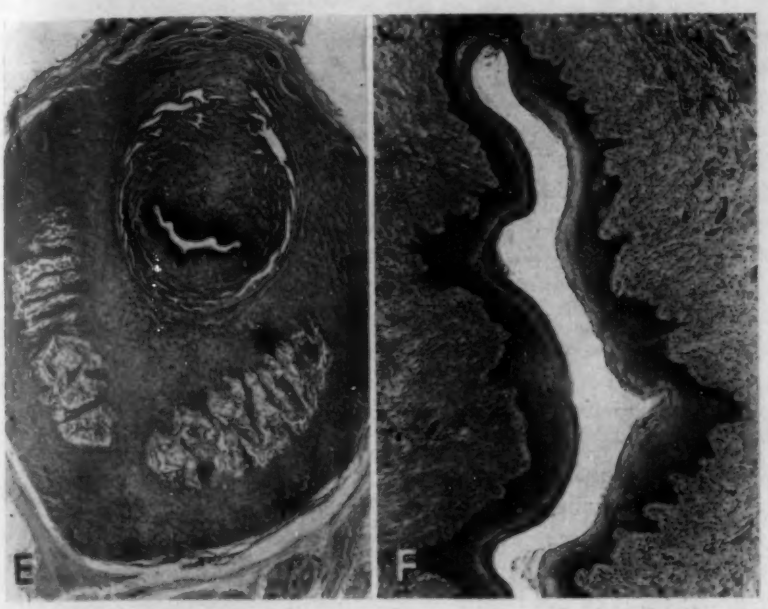


Fig. 3.—*A*, cross section of the penis of a control adult dog, showing an island of bone; *B*, epithelium of the penile urethra of a control adult dog; *C*, cross section of the penis of an adult dog which had received 396,000 rat units of estradiol benzoate over a period of five months, showing irregularity in the contour of the bone (arrow); *D*, keratinized epithelium of the penile urethra of the dog mentioned in *C*; *E*, cross section of the penis of an adult dog which had received 485,000 rat units of estradiol benzoate over a period of seven and one-half months, showing absence of bone; *F*, extreme keratinization of the epithelium of the penile urethra of the dog mentioned in *E*.

When the experiments were terminated at the end of the stated periods, a roentgenographic examination of the entire skeleton of each dog was made, and subsequently many of the long bones were halved and studied roentgenographically again. Sections from the following regions were studied microscopically: costochondral junction, humeroscapular articulations, knees, hips, pubic symphysis, sacroiliac articulations, calvarium and lumbar vertebrae. Some of the nonosseous tissues were also examined microscopically.

OBSERVATIONS

The dogs which received injections of estradiol benzoate were considerably shorter and lighter in weight than the controls. The roentgenographic examination demonstrated that some of the long bones and the snouts were shorter in the experimental animals than in the controls. The difference in length of the corresponding long bones was from 1/16 to 1/2 inch (0.16 to 1.27 cm.); the snout, too, was about 1/4 to 1/2 inch

(0.64 to 1.27 cm.) shorter in the experimental animals. After a seven and one-half month period of injection of the estrogen, the only epiphysial plate still partially open in the long bones as viewed roentgenographically was that in the upper end of the humerus. In the control animals observed for a corresponding period, the epiphysial plates in the upper ends of the humerus, tibia and femur were open. The heights of the vertebral bodies and the sternum were shorter in the experimental animals. In addition, all the long bones of these animals presented some narrowing proportional to the stunting of longitudinal growth. In contrast to what is found in mice, no evidence of osteosclerosis was observed, in either the metaphysial or the endosteal regions; furthermore, the density of the calvarium was unaffected (figs. 1 and 2).

The pubic bones of the dog subjects showed roentgenographically no changes similar to those induced in guinea pigs and mice by estrogens.[4] The normal center of ossification in the lowermost portion of the pubic symphysis was merely smaller in the experimental than in the control dogs. The sacroiliac regions revealed nothing unusual.

On the other hand, the roentgenograms showed that the penis of the experimental animal had only a small proximal segment of bone, while the penis of the normal animal had a long stem of bone running almost throughout its length (figs. 1 *E* and 2 *E*).[5]

The microscopic study confirmed for the most part the findings noted roentgenographically. It showed plainly that the epiphysial plates of the examined long bones of the experimental animals were closed. The costochondral junctions and the growth plates in the pubic symphysis were found only slightly affected, in that the number of hypertrophic cells was diminished in them. The articular cartilages of the long bones were slightly thinner and contained fewer cells in the experimental animals than in the controls. Nowhere was there any evidence of premature fibrillation or degeneration of the cartilage matrix. Furthermore, osteosclerosis was not noted in the metaphysial or the endosteal areas. The bones and ligaments comprising the pubic symphysis and the sacroiliac articulations were unaffected.

As far as the nonosseous tissues are concerned, in addition to the well known changes produced by estrogens in the prostate and in the uterus, cervix and vagina, keratinization was observed in the epithelium of the penile urethra, and adenomas were seen in the cortices of the adrenal glands (fig. 3). The pituitary glands (examined by A. E. Severinghaus) showed absence of the cellular changes usually noted in these glands after the administration of an estrogen.

---

4. Gardner, W. U.: Am. J. Anat. **59**:459, 1936. Sutro, C. J., and Pomerantz, L.: Arch. Surg. **39**:992, 1939.

5. Sindram, I. S.; Van der Woerd, L. A., and De Jongh, S. E.: Acta Neerland. Morphol. **2**:236, 1939.

SUMMARY

The prolonged administration of estradiol benzoate to young mongrel dogs did not cause osteosclerosis. Inhibition of the growth of the skeleton and disturbance of the development of bone in the penis, however, did occur. No changes were evident in the pubic symphysis or in the sacroiliac joints. These findings contrast in part with those noted as occurring in certain strains of young mice, in which not only inhibition of skeletal growth but also osteosclerosis resulted from treatment with estrogens. The absence of osteosclerosis in the dogs suggests that other factors besides the inhibition of growth may be responsible for the excess production of bone in certain animals after the administration of estrogen.

REACTION OF BONE TO METASTASIS FROM CARCINOMA OF THE BREAST AND THE PROSTATE

WENDELL S. SHARPE, M.D.
Fellow in Surgery, Mayo Foundation
AND
JOHN R. McDONALD, M.D.
ROCHESTER, MINN.

That metastatic carcinoma of the breast and that of the prostate, of all types of tumor, most widely involve the osseous system is a matter of common consent. However, as yet no satisfactory or plausible explanation has been advanced for the marked difference in type of reaction of bone encountered in cases of metastasis from carcinoma of either of these two organs. Roentgenologically, two main types of lesions are described, roughly designated as osteoplastic and osteoclastic. In cases of carcinoma of the prostate the osseous metastatic growths are usually osteoplastic. In cases of metastasis from carcinoma of the breast, the bony lesions are usually osteoclastic. Mixed lesions occur in but a small percentage of cases. This marked, well established difference in the reaction of bone to metastasis has prompted this study. No attempt has been made to investigate the chemical reaction of bone, although in this field probably lies the ultimate answer to the question.

As early as 1834 or about the time of the inception of cellular pathology, Sanson [1] described a case of pathologic fracture of the femur in a woman suffering from scirrhous carcinoma of the breast. Twenty years later the first case of osseous metastasis from prostatic carcinoma was described by Thompson.[2]

There have been many studies of the osteoplastic reaction of bone to metastatic carcinoma and nearly as many concerning the osteoclastic reaction. In an attempt to explain the osteoplastic reaction in osseous metastasis from carcinoma three main hypotheses have been developed: (1) that the formation of new bone is secondary to circulatory disturbances produced by the metastasis, (2) that the formation of new bone is of the character of a foreign body or defense reaction, and (3) that the metastasizing cells themselves secrete a substance stimulating osteo-

---

From the Section on Surgical Pathology, Mayo Clinic.

1. Sanson, cited by Piney, A.: Brit. J. Surg. **10**:235, 1922.
2. Thompson, H.: Tr. Path. Soc. London **5**:204, 1854.

genesis. The first hypothesis was propounded by von Recklinghausen,[3] who showed that osseous metastasis occurred by way of the vascular channels of the marrow. He advanced the opinion that the resultant stagnation of blood presented a condition conducive to formation of new bone. This hypothesis of vascular stagnation is based on the fact that the vascular channels of bones are relatively wide and are of fixed caliber. Von Recklinghausen maintained that any osseous new growth in response to carcinomatous cells was the result partly of chronic venous congestion and partly of reactive irritation at the edges of the metastatic deposits. The second hypothesis of the formation of new bone in metastatic osseous lesions is based on the latter factor. Von Recklinghausen expressed the opinion that the amount of the reaction in bone depended to some extent on the reaction of the tissues in the primary tumor.

The third school of thought has as its exponents workers in the field of chemistry. One of the leaders along this line is Kay,[4] who in 1932 published a scholarly article on the action and mechanism of phosphatase in growth and disease of bone. Gutman, Sproul and Gutman [5] showed that normally in the lumbar vertebrae, the pelvis and the ribs there is an appreciable amount of phosphatase activity; further, that this activity is increased greatly in osteoplastic metastasis while it is decreased to less than the level of normal in the presence of osteolytic processes. They reasoned that in normal bone the intercellular fluids bathing osteogenic cells contain salts of phosphoric esters, which are hydrolyzed by the phosphatase elaborated by those cells. They expressed the opinion that the capacity of certain metastatic neoplasms in bone to stimulate the production of phosphatase in bone determines osseous growth in part, so that the estimated high phosphatase content of neoplastic tissues in osteoplastic metastasis conceivably is related to increased osteogenesis.

Geschickter [6] expressed the opinion "that the type of metastasis is dependent on the path of its entrance into bone." When the cells are borne by the blood, osteolysis results, according to his belief. When "tumor reaches bone through lymphatic permeation, osteoplasia follows." Although some observers have denied the existence of lymphatics in bone marrow, the presence of such channels was proved conclusively by

3. von Recklinghausen, F.: Die fibröse und deformirende Ostitis, die Osteomalacie und die osteoplastische Carcinose in ihren gegenseitigen Beziehungen, in Festschr. Rudolph Virchow zu seinem 71. Geburtstage gewidmet von den früheren und jetzigen Assistenten des Berliner pathologischen Instituts, Berlin, G. Reimer, 1891.

4. Kay, H. D.: Physiol. Rev. **12**:384, 1932.

5. Gutman, E. B.; Sproul, E. E., and Gutman, A. B.: Am. J. Cancer **28**: 485, 1936.

6. Geschickter, C. F.: Radiology **16**:111, 1931.

Kolodny,[7] who injected india ink into the medullary cavity of a bone and found it five to seven days later in the lymph nodes in that region.

Much of the investigation on osteoplasia in bone as a result of metastasis applies to destruction of bone in metastasis. The consensus is that destruction of bone as the result of metastasis of carcinoma occurs through two processes: 1. The carcinomatous cells themselves appear to have the faculty of directly eroding bone. 2. There is increased osteoclastic action in the vicinage of carcinomatous deposits, probably because of some unknown stimulus provided the osteoclasts by the carcinomatous mass.

METHOD OF STUDY

The clinical portion of this study is based on 415 cases of primary carcinoma of the breast and 106 of primary carcinoma of the prostate. In every instance the primary neoplasm was available for histologic study. Of the 415 cases of carcinoma of the breast, osseous metastasis was shown by roentgenologic study to have developed in 219. Specimens of the osseous lesions were available for study in 23 cases. As a comparison the cases of 196 patients were taken who had had primary carcinoma of the breast, who were living and well an average of nineteen and nine-tenths years after the surgical removal of the carcinoma and who did not show any roentgenologic evidence of metastasis.

Of the 106 cases of carcinoma of the prostate, there were 66 in which roentgenologic examination revealed osseous lesions. In 16 cases specimens of the osseous metastatic growths were available for study. A series of 40 cases of primary carcinoma of the prostate was taken as a control group. All patients of the control group were well and free of symptoms with no evidence of metastasis a minimum of five years and a maximum of eighteen years after the recognition of the carcinoma.

The fixed frozen tissue technic was employed, the sections being stained with hematoxylin and eosin, and each tumor was graded by the method of Broders.[8]

The technic employed in the decalcification of blocks of bone was that described by De Galantha[9] in 1937. The specimens of bone were studied in the gross, and blocks of the region containing a metastatic growth were cut by a band saw. In addition to staining sections with hematoxylin and eosin, we used Van Gieson's stain on all specimens.

CLINICAL STUDY OF OSSEOUS METASTATIC GROWTHS

Formation of Bone.—In this series in most cases of carcinoma of the breast (97.3 per cent) there were osteoclastic lesions in the osseous metastatic growth, while in most cases of prostatic carcinoma (97 per cent) there were osteoplastic lesions in the bony metastatic growth (table 1).

7. Kolodny, A.: Arch. Surg. **11**:690, 1925.
8. Broders, A. C.: Minnesota Med. **8**:726, 1925.
9. De Galantha, E.: Am. J. Clin. Path. (Tech. Supp.) **7**:10, 1937.

Age Incidence in Regard to Probable Metastasis.—The age at which the tumor, whether of the breast or of the prostate, was discovered and at which operation was performed, does not appear to be a factor influencing the probability of metastasis. The average age at time of operation (46 years for breast; 63 years for prostate) was almost identical in the control groups and in the groups in which there was metastasis. There is no definite proof from this study that, exclusive of the grade

TABLE 1.—*Types of Osseous Lesion in Metastasis of Carcinoma of the Breast and Carcinoma of the Prostate as Evident in the Roentgenogram*

	Breast		Prostate	
Type	Cases	Per Cent	Cases	Per Cent
Osteoclastic	213	97.3	2	3.0
Osteoplastic	6	2.7	64	97.0
Total	219	100.0	66	100.0

TABLE 2.—*Histologic Grade (Broders' Method) of Carcinoma of the Breast and Its Relation to Osseous Metastasis*

	Grade 2		Grade 3		Grade 4		
	Cases	Per Cent	Cases	Per Cent	Cases	Per Cent	Total Cases
No metastasis	47	24.0	75	38.3	74	37.7	196
Metastasis	30	13.7	73	33.3	116	53.0	219

TABLE 3.—*Histologic Grade (Broders' Method) of Carcinoma of the Prostate and Its Relation to Osseous Metastasis*

	Grade 2		Grade 3		Grade 4		
	Cases	Per Cent	Cases	Per Cent	Cases	Per Cent	Total Cases
No metastasis	10	25.0	21	52.5	9	22.5	40
Metastasis	24	36.4	25	37.9	17	25.7	66

of malignancy of the carcinoma, the younger the person at the time of operation the more certain or quicker the metastasis.

Grade of Malignancy of the Primary Tumor in Relation to Metastasis.—Tables 2 and 3 demonstrate the findings in this study. There appears to be less tendency for a tumor of low grade malignancy in the breast to metastasize to bone. Eighty-six per cent of the tumors of the breast which metastasized to bone are of grade 3 or grade 4 malignancy histologically. On the other hand, there does not appear to be any

greater tendency for a tumor of grade 4 malignancy in the prostate to metastasize than for one of grade 2 malignancy in the prostate.

Factors Supposedly Influencing Metastasis.—There are many who believe that the ability or tendency of any tumor to metastasize is

TABLE 4.—*Carcinoma of Breast—Elapsed Time from Discovery of Tumor to Operation*

	196 Cases—No Metastasis	215 Cases—Metastasis *
Longest	132 months	132 months
Shortest	2 weeks	1 month
Average	11.06 months	11.06 months

* In 4 of the original 219 cases the lesion was considered inoperable.

TABLE 5.—*Carcinoma of Prostate—Elapsed Time from Onset of Symptoms to Operation*

	40 Cases—No Metastasis	60 Cases—Metastasis *
Longest	74 months	118 months
Shortest	1 month	1 month
Average	24.1 months	21.8 months

* In 6 of the original 66 cases operation was not performed—metastasis was demonstrated at necropsy.

TABLE 6.—*Relation of Biopsy and Trauma to Osseous Metastasis in Carcinoma of the Breast*

	196 Cases—No Metastasis		219 Cases—Metastasis	
	Number	Per Cent	Number	Per Cent
Biopsy	11	5.6	20	9.1
Trauma	14	7.1	20	9.1
Treatment other than surgical	12	6.1	20	9.1

TABLE 7.—*Relation of Biopsy and Trauma to Osseous Metastasis in Carcinoma of the Prostate*

	40 Cases—No Metastasis		66 Cases—Metastasis	
	Number	Per Cent	Number	Per Cent
Biopsy	1	2.5	10	15.2
Trauma	12	30.0	8	12.1
Treatment other than surgical	0	0	4	6.1

inherent in the tumor and is not influenced by time, biopsy, trauma or other extraneous factor. In that regard tables 4, 5, 6 and 7 are of great interest. The elapsed time from onset of the symptoms in prostatic carcinoma to operation and the elapsed time from discovery of the tumor in carcinoma of the breast to operation are practically identical in the

group in which there was metastasis and in the control group. Furthermore, biopsy, trauma and treatment other than proper surgical intervention do not influence the probability of ensuing osseous metastasis greatly (tables 6 and 7). The term "biopsy" is self explanatory. By "trauma" is meant physical violence, massage or manipulation. Treatment other than operation includes chemical applications, plasters, diathermy, electric treatment, and so forth. However, in regard to carcinoma of the breast, in only 37 of 196 cases (19 per cent) without metastasis had there been biopsy, trauma or treatment other than surgical, while in only 60 of the 219 cases (27 per cent) in which osseous lesions occurred was there a record of them. In more than 5 per cent of the control group, biopsy had been performed an average of two months before operation.

Lymphatic Involvement in Relation to Osseous Lesions.—It is well known that involvement of the skin and lymph nodes at the time of mastectomy shows spread of the disease. The prognostic value of these signs as far as it concerns later osseous involvement is shown by the fact that in only half of the control group was there such spread of the disease, whereas in 75 per cent of the group in which there was metastasis to bone there was also involvement of both lymph nodes and skin. Furthermore, as far as osseous metastasis is concerned, it appeared to make little difference whether skin or lymph nodes or both were involved. Those growths the malignancy of which was graded 4 and which had involved the skin or lymph nodes were nearly twice as likely to metastasize to bone, however, as growths of similar malignancy which had not involved the skin or lymph nodes.

Elapsed Time from Operation to Onset of Osseous Symptoms.—The control group of 196 patients who had carcinoma of the breast were observed for an average of nineteen and nine-tenths years. All the patients were free of symptoms and healthy and were without evidence of metastasis. The longest period of observation was twenty-seven years; the shortest, thirteen and five-tenths years. Among the patients who had carcinoma of the breast with metastasis, symptoms suggesting osseous metastasis were observed first an average of fifteen months after operation or about twenty-six months after discovery of the tumor. At this time it was frequently impossible to confirm the metastasis by roentgenologic examination. In a few of the patients, however, it was ten to thirteen years before the onset of symptoms of metastasis. Usually another ten to fifteen months elapsed after the onset of the symptoms referable to osseous metastasis before confirmation was obtained by roentgenologic examination.

The control group of 40 patients who had prostatic carcinoma were observed for an average of nearly ten years. The longest period of observation was nineteen years and the shortest five years. In carcinoma of the prostate osseous involvement may be already present at the time of operation for relief of urinary obstruction. Seven such cases were among the 66 cases studied in which metastatic growths were evident. In this group the longest period from onset of symptoms of carcinoma of the prostate to onset of distress referable to osseous metastasis was nearly ten years. There seemed to be no definite time at which osseous symptoms appeared. However, the average of about two years from the onset of symptoms of prostatic carcinoma agrees with the results of most authors.

PATHOLOGIC STUDY OF THE OSSEOUS LESIONS

As has been stated, the metastatic lesions in bone were studied in 16 cases in which the primary lesion was a carcinoma of the prostate and in 23 cases in which the primary lesion was a carcinoma of the breast.

Prostate.—Of the 16 cases of metastatic carcinoma in which the primary lesion was in the prostate, 15 presented an osteoplastic appearance of the osseous lesion under roentgenologic examination; in the sixteenth case the bone lesion was of the mixed type. On gross examination the metastatic lesion usually appeared as a grayish or grayish pink nodule in the bone. Less frequently, no definite mass of tumor tissue could be seen, but the involved bone was hard and white or whitish yellow. Frequently, particularly in the bones of the pelvis, the neoplasm produced elevation of the periosteum. This tumor tissue when present as a circumscribed mass was softer than normal bone and easily compressed by the finger. In 1 or 2 cases, large hematomas were found in tumors. The second type, in which the bone was hard and whitish, occurred most commonly in the long bones.

The grade of malignancy of the primary carcinoma was reproduced in the metastatic lesion in every instance in this series. It would appear that the grade of malignancy of the carcinoma had no effect on the amount of new bone formed since similar changes were found with carcinoma in which there was considerable differentiation as well as with carcinoma in which the cells were very anaplastic.

The inception of the metastasis in most cases seemed to be in the marrow, in which small emboli of carcinomatous cells frequently could be demonstrated in the vessels surrounded by normal bone. Infarcts were quite numerous throughout the involved portion of bone, and these infarcts usually were associated with hemorrhage (fig. 1 *a*). Hemorrhagic infarcts frequently were associated with large masses of tumor cells, and in such regions unusual osteoblastic activity with formation

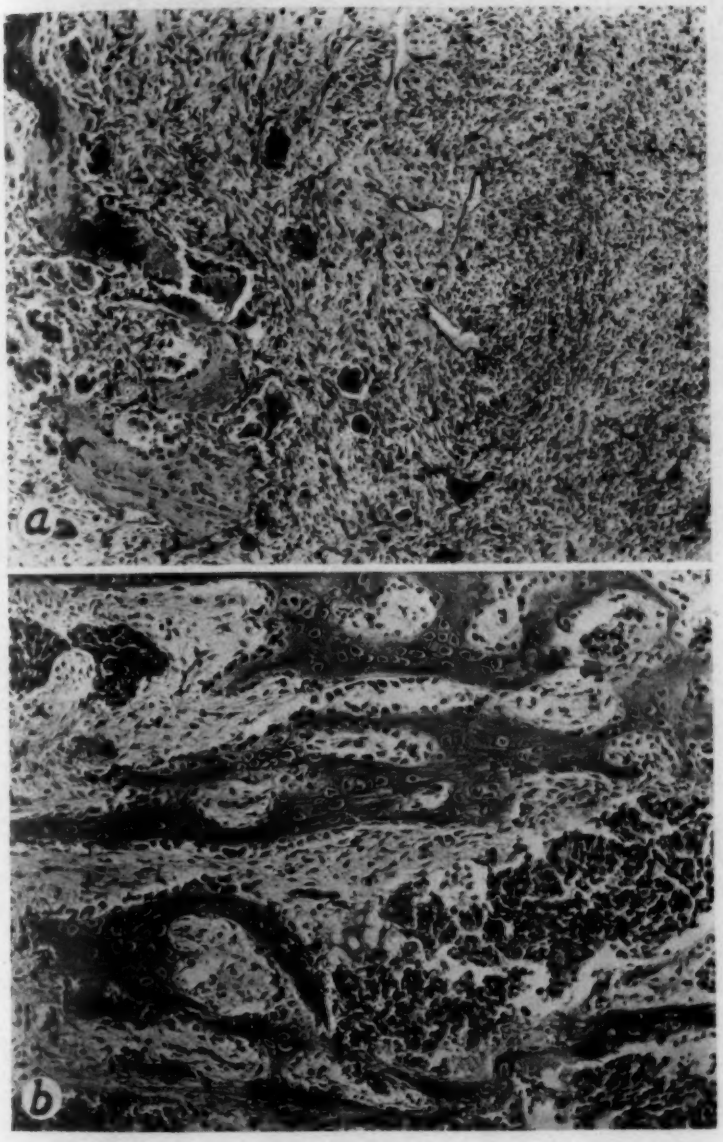


Fig. 1.—Metastasis from primary carcinoma of the prostate: (*a*) In rib. The edge of an infarct is shown with a few islands of carcinomatous cells and considerable fibrous tissue. Notice the osteoid tissue or new bone and active osteoblasts (hematoxylin and eosin; × 100). (*b*) In ilium. There is a large quantity of new bone surrounded by fibrous tissue. The osteoblasts are large and active. Around the islands of carcinomatous cells there is very little formation of new bone (hematoxylin and eosin; × 100).

of new bone was apparent. In every instance, however, in addition to the formation of new bone, destruction of bone could be found.

The amount of growth of new bone seemed to bear a definite relation to the amount of fibrous tissue formed around the carcinomatous cells. In those regions in which there was much formation of new bone, there was also much fibrous tissue and vice versa (figs. 1 *b* and 2 *a*). Collagen fibrils were formed around the carcinomatous cells and tended to enmesh them. Ranged along the collagen fibrils at intervals, osteoblasts could be demonstrated forming new bone.

In the single case in which mixed osteoclastic and osteoplastic metastasis could be demonstrated, a similar appearance to what has been described already could be found in the osteoplastic regions. However, in the osteoclastic regions great masses of tumor cells were apparent surrounded by little fibrous tissue. Osteoblastic activity, as evidenced by osteoid tissue or new bone, also could be demonstrated. However, in this case the predominant feature was destruction of bone.

In some regions small groups of carcinomatous cells were found in the marrow adjacent to the endosteum. From such a source it appeared that the trabeculae of new bone arose as a result of stimulation of endosteal osteoblasts by carcinomatous elements. These new trabeculae then grew into the masses of tumor cells and segregated them into groups.

There were many regions in which cancer cells abounded but in which new bone was not seen. At first this was puzzling, but comparison with those regions in which proliferation of bone was occurring revealed the absence in the first instance of any connective tissue elements in the vicinity of these tumor cells. As a result, in these regions there was no transformation of fibroblasts to osteoblasts with the production of osteoid tissue.

In some cases osteoplastic growth was noticeable beneath the periosteum. The connective tissue bundles in the periosteum were invaded by carcinomatous cells. In some sections, in which osteoclastic activity was conspicuous, a few osteoclasts were present. However, osteoclasts were found associated with destruction of bone so infrequently that they do not offer an adequate explanation for osteoclasia in metastatic malignant lesions.

Breast.—There were 23 cases of carcinoma of the breast in which the osseous metastatic growths were available for study. In 22 of these cases the growth was frankly osteoclastic in type, while in 1 instance roentgenologic examination showed a plastic growth. In most cases the medullary cavity was replaced by tumor tissue, which appeared grayish to the naked eye. The cortex of the bone on either side of the mass appeared to be thinner, and it was possible to fracture it easily. The

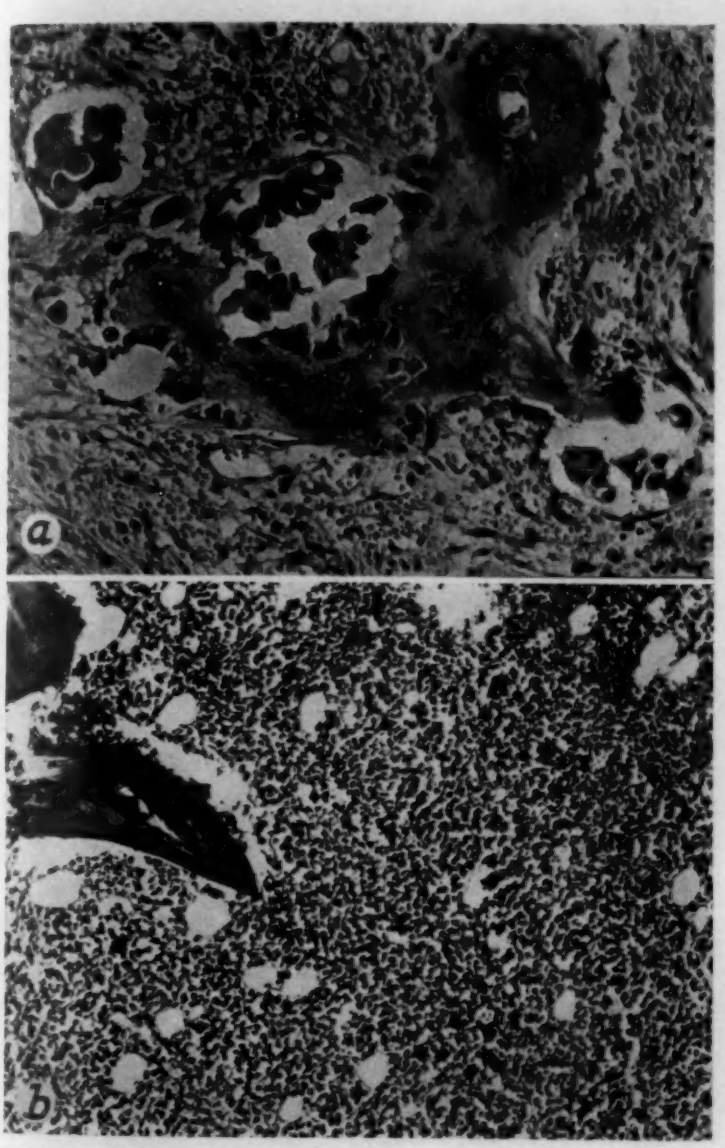


Fig. 2.—(*a*) Metastasis in rib from primary carcinoma of the prostate. An island of osteoid tissue (new bone) is shown surrounded by young fibroblasts. Several islands of carcinomatous cells are shown, and in these regions the osteoid tissue is being eroded (hematoxylin and eosin; × 180). (*b*) Metastasis in vertebra from primary carcinoma of the breast. There is extensive osteoclastic activity, only a few spicules of old bone remaining. Note the absence of fibrous tissue (hematoxylin and eosin; × 100).

neoplastic mass in the bone was soft and easily compressed and usually demonstrated rather definite margins. Occasionally an infiltrating type of lesion was found in which the tumor cells could be demonstrated at quite a distance from the gross limits of the metastatic lesion. Hemorrhage was encountered much more frequently than in the metastatic lesions from carcinoma of the prostate. Elevation of the periosteum by the expanding tumor mass was seen, especially in cases in which a rib, the sternum or a clavicle was the bone involved. In none of these nodular growths could any bony proliferation, subperiosteal or otherwise, be seen grossly. Of all bones studied, those of the pelvis seemed to exhibit lesions less well circumscribed than others. The long bones, such as the humerus and the femur, usually were found to be involved with well circumscribed, well delineated lesions.

As in the prostatic lesions, the grade of malignancy of the primary lesion was reproduced in every instance in the bony metastasis. It did not appear that the amount of fibroplasia or osteoclasia was influenced by the relative differentiation or dedifferentiation of the tumor.

In the majority of these sections one of the most consistent observations was the apparent destruction of both spongy and cortical bone by direct contact of the tumor cells with the bone (fig. 2 *b*). These tumor cells were arranged variously, often as nests, frequently as solid masses of epithelial cells. Only in a few instances and not consistently was it possible to demonstrate fibrous tissue interposed between the tumor and the bone, as so often occurred in metastasis from carcinoma of the prostate. Osteoclasts were slightly more numerous in and surrounding the lesions than in the similar sections of bone in cases in which carcinoma of the prostate was the primary lesion. Here again osteoclasts did not appear to be numerous enough to account for all the destruction of bone.

In many regions, apparent infarction had taken place. Hemorrhagic changes frequently resulted from the infarction. In about half the cases studied, the metastatic process had originated in the medullary spaces.

In some places, the haversian canals were filled with tumor tissue, and the bony trabeculae were surrounded by bony cells. In all these the tumor cells alone were so often the center of destruction of bone that it must be concluded that they acted in the capacity of osteoclasts. In every section of bone containing a metastatic growth from carcinoma of the breast a few strands of fibrous tissue could be demonstrated, and here again the evidence of fibroblastic metaplasia to osteoblasts could be found (fig. 3 *a* and *b*). However, destruction of bone was so overwhelming as to render this small amount of formation of bone negligible.

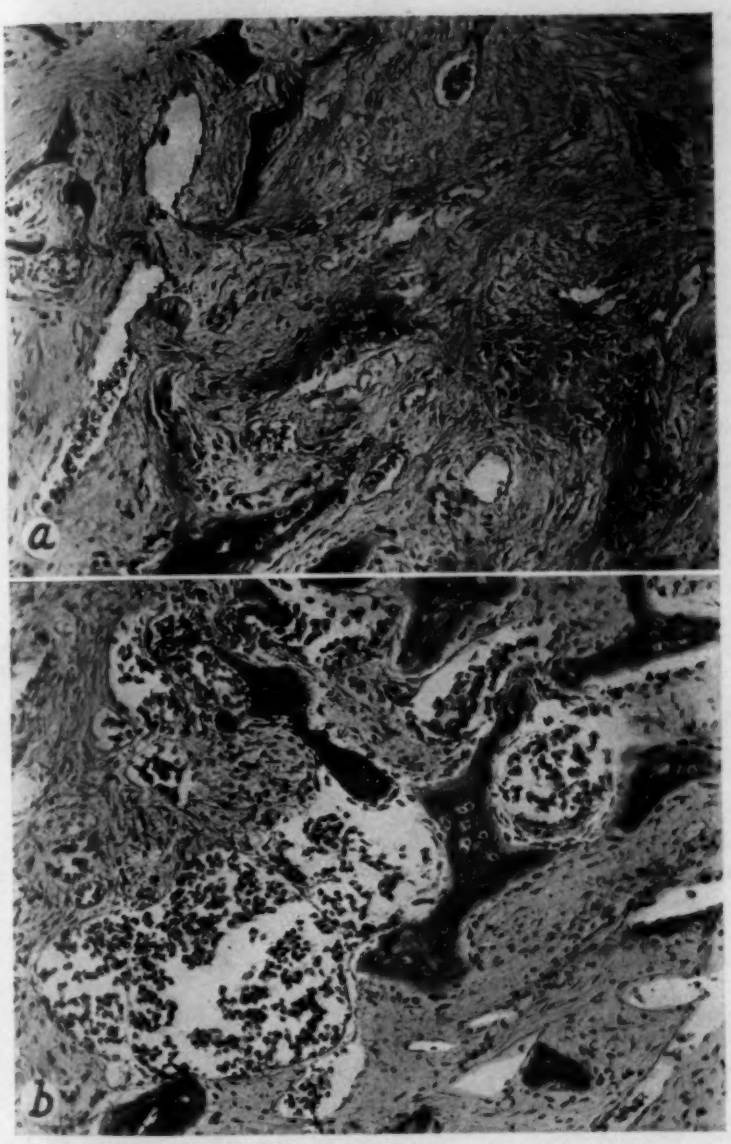


Fig. 3.—Metastasis in the femur from primary carcinoma of the breast: (*a*) In this region there is osteoplastic activity. The osteoid tissue with active osteoblasts is seen readily. Carcinoma cells can be seen in vessels (hematoxylin and eosin; × 100). (*b*) Both osteoplastic and osteoclastic activity are shown. In the osteoclastic regions the carcinoma cells are in direct contact with the bone (hematoxylin and eosin; × 100).

COMMENT

The conclusion that biopsy, trauma or treatment other than surgical does not predispose greatly to osseous metastasis suggests an inherent capacity of tumors to metastasize to bone. This capacity may be similar to that which causes the metastasis to be either osteoclastic or osteoplastic.

Although metastatic lesions in bone produce either an osteoplastic or an osteoclastic reaction, rarely a mixture from a roentgenologic aspect, it has been shown that microscopically it is always possible to recognize the two processes of formation and of destruction of bone in any given lesion. In the osseous metastasis from carcinoma of the prostate, the predominant effect is formation of bone, while destruction of bone is the outstanding microscopic feature in the bone containing a metastatic lesion from carcinoma of the breast. The evidence seems to indicate that microscopically identical tumor cells in the osseous metastatic growths from different primary sites (prostate and breast) have the faculty, on the one hand, of stimulating growth of new bone and, on the other hand, of suppressing osteogenic activity. The mechanism of this process is obscure but it is probably chemical. However, it has been noted that much more fibrous tissue is formed in the bone which is the site of metastasis from carcinoma of the prostate than in a bone which is the site of metastasis from a carcinoma of the breast. Furthermore, the fibrous tissue in a bone in which there is carcinoma which has metastasized from the prostate shows an active tendency to form osteoid tissue and bone, apparently by the transition of fibroblasts to osteoblasts. On the other hand, less fibrous tissue is formed around the carcinomatous cells in bone from a primary lesion in the breast. In addition, the tumor cells which had metastasized from carcinoma of the prostate appeared to stimulate the endosteal as well as the subperiosteal osteoblasts to activity, a feature which is minimal or absent in bony metastasis from carcinoma of the breast.

SUMMARY

In 97.3 per cent of the cases in which the primary carcinoma was in the breast there was evidence of osteoclasis in the bony metastasis while in 97 per cent of cases in which the primary carcinoma was in the prostate there was osteoplasia in the bony metastasis. Histologically, the study of the lesion showed that although the predominant structural change in bone was either destruction of bone or formation of new bone, depending on the site of the primary lesion, yet it was always possible to demonstrate both processes in the same lesion. In the osseous metastatic growths from carcinoma of the prostate, large amounts of

fibrous tissue were observed almost invariably, which could be demonstrated to be undergoing a transformation into osteoid tissue. Fibrous tissue was seen in smaller quantities in the osseous lesions which resulted from carcinoma of the breast. The histologic studies in cases in which the primary lesion was in the breast revealed that the growths of the higher grades of malignancy showed more tendency to metastasize to bone than those of the lower grades. Little difference was observed between the different grades of carcinoma of the prostate in tendency of the growth to metastasize to bone.

EVOLUTION OF EXPERIMENTAL RADIATION ULCERS OF THE INTESTINE

NATHAN B. FRIEDMAN, M.D.*
AND
SHIELDS WARREN, M.D.
BOSTON

It is generally known that ulceration of the intestine occurs as a complication of radiation therapy in human patients [1] and that comparable lesions have been produced in experimental animals by many workers.[1b] There is, however, no general agreement as to the genesis of such lesions.[1b]

The present report is one of a series dealing with various aspects of the effects of radiation on the intestinal tract. It is concerned primarily with the pathologic changes occurring during the evolution of radiation lesions in the intestines of rabbits.

METHODS

The observations were made on 32 rabbits. They received a course of roentgen radiation totaling 4,000 roentgens (r) (measured in air), distributed in eight or ten doses of 400 or 500 r each, given approximately every other day. A single anterior abdominal portal 10 by 15 cm. in size was irradiated. The upper limit of the field was along the lower border of the costal margin, with the liver and stomach shielded. The technical conditions were: 140 kilovolts; 8 milliamperes; 32.5 cm. target skin distance; filtration—0.25 mm. copper, 2 mm. aluminum and 3 mm. celluloid. The rabbits' bodies were approximately 10 cm. thick when stretched in position for irradiation. The depth doses were 60 per cent at 5 cm. and 30 per cent at 10 cm.

The animals were killed at various periods of time after the completion of the course of treatment, ranging from one week to six months. Several animals were killed in the two to three week period during which radiation was being administered. Tissues were fixed in Zenker's fluid and stained with eosin and methylene blue, phosphotungstic acid–hematoxylin, Mallory's connective tissue

---

* Littauer Fellow in Pathology.

From the Laboratories of Pathology of the New England Deaconess Hospital and the Collis P. Huntington Memorial Hospital and the Department of Pathology, Harvard Medical School.

1. (*a*) Warren, S., and Friedman, N. B.: Pathology and Pathologic Diagnosis of Radiation Lesions in the Gastro-Intestinal Tract, to be published. (*b*) Friedman, N. B.: The Stomach and Intestine, in Warren, S.: The Effects of Radiation on Normal Tissues, Arch. Path., to be published.

stain, Weigert's elastic stain and the Foot stain for reticulin. Routine sections were taken from the jejunum and ileum, including the lymphoid ileocecal junction (sacculus rotundus), the cecal appendage, the appendix, and the ascending and descending colon. Additional sections were taken from all grossly abnormal sites, such as ulcers.

PATHOLOGIC OBSERVATIONS

Emphasis will be placed on the pathologic changes in the mucosa since our observations indicate that ulceration developed as a consequence of the primary radiation damage to the structures of the mucous membrane. Several distinct reaction patterns of the mucosa fall into

Summary of Observations

Time	Musosa	Other Tissues
During the radiation period		
2 to 3 weeks.....	Nuclear swelling Inhibition of mitosis Mucous change	Edema Telangiectasia Destruction of lymphoid tissue
After radiation		
1st week.....	Mitotic activity Epithelial abnormalities Mucosal atrophy Erosion Ulceration (colon)	
2d week.....	Regeneration (small intestine) Persistent atrophy and ulceration	Persistent edema and ectasia
3d and 4th weeks.....	Regeneration (colon) Persistent atrophy and ulceration	
2d month.....	Repaired small intestine Persistent ulceration	Regeneration of lymphoid tissue
3d month.....	Repaired colon Persistent ulceration	Early hyalinization Repaired lymphoid tissue
3d to 6th month.....	Residual cystic glands Focal mucous stasis	Marked hyalinization Persistent ectasia

chronologic stages with considerable overlapping (table). There was an initial epithelial cellular reaction consisting of nuclear swelling, inhibition of mitosis and mucous change. This was followed by mucosal atrophy and later by regeneration, both of which proceeded side by side and were accompanied by progressive ulceration and inflammation. The final healed stage showed the residua of reaction.

Mucosal Changes.—(*a*) Initial Epithelial Reaction: Changes in the epithelial cells were seen during the first few days of the course of radiation. No mitotic figures were present in the crypts, and the cell nuclei were markedly swollen with prominent owl's eye nucleoli and clumped chromatin. The altered crypt cells were in striking contrast to

the epithelial lining of the villi and surface, the cells of which appeared normal. This radiation effect was more marked in the small intestine than in the colon.

After a few days the so-called mucous change was present. This consisted in stasis of mucus in the goblet cells of the large intestine and appearance of increased numbers of mucous cells in the small intestinal crypts. Overproduction of mucus was evident grossly, and mucous casts were seen. As will be demonstrated later, focal mucous stasis in the colon persisted for months. Toward the end of the two to three week radiation period, mitotic figures were seen, some of them abnormal ones, and the cells in the crypts showed atypical nuclei and distorted cell bodies. The nuclei were swollen and bizarre, while the cells were cuboidal or flattened and were irregularly arranged.

(*b*) Atrophy: After one week the glands and villi became less prominent and almost disappeared in extreme instances. Some gland remnants were cystic. The surface epithelium consisted of a cuboidal, basophilic, undifferentiated cell layer with variation in size and shape from cell to cell. Vacuolated owl's eye nucleoli were present. The bulk of the mucosa was made up of edematous connective tissue with inflammatory cell infiltration. Polymorphonuclear leukocytes and pseudoeosinophils were first seen, but later lymphocytes and mononuclear leukocytes predominated and persisted for weeks. Although these changes were present in the small intestine to a minor degree, they were quickly overshadowed by regenerative phenomena. In the colon a persistently atrophic mucous membrane was present for weeks before adequate repair set in.

(*c*) Regeneration: In the atrophic mucosa, with its thickened and infiltrated stroma, hyperplastic glandular structures appeared. They showed papillary projections, numerous mitotic figures, and mucous cell types, some abnormal. Regeneration was more active in the small intestine than in the colon and appeared relatively early, sometimes as soon as two weeks after treatment with radiation. Regeneration in the colon usually did not begin until after three to four weeks, and sometimes considerably later. The hyperplastic glandular structures in the colon were rich in mucous cells. Repair of the mucosa of the colon was slower than that of the small intestine. The latter often returned to nearly normal in the second month, while the former frequently required a month longer.

(*d*) Residua: From the third to the sixth month the mucosa throughout most of the intestinal tract returned almost entirely to normal. In the presence of ulcers, however, the bordering mucosa showed considerable atrophy despite a more normal picture elsewhere. There were also other changes that appeared to be residua of earlier reactions. The

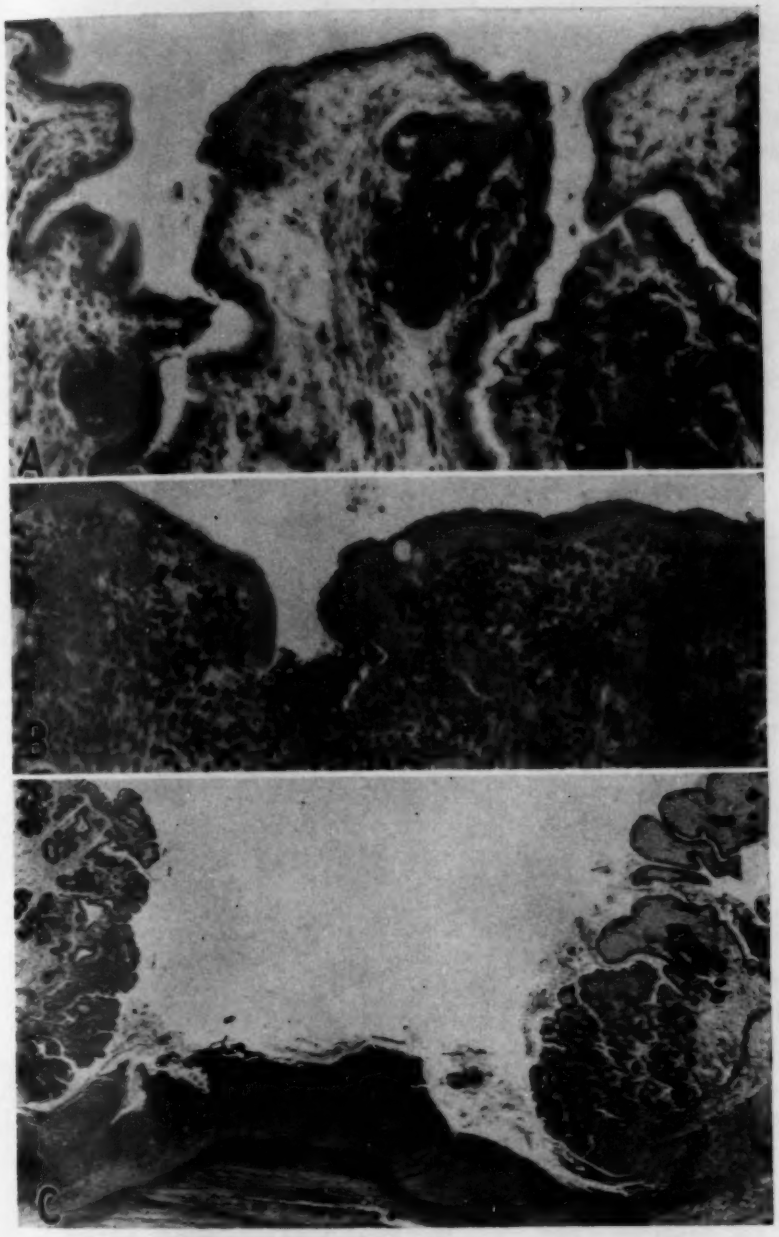


Fig. 1.—*A*, colon first month after irradiation, showing atrophic mucosa with some regeneration (× 100).

A, colon first month after irradiation, showing atrophic mucosa with some regeneration (× 100).

B, colon first month after irradiation, showing atrophic mucosa with early ulceration and inflammation (× 150). Note the abnormal cells and glands in the mucosa.

C, colon third month after irradiation, showing advanced ulceration in an abnormal mucosa (× 14).

most striking was the presence of an occasional focus of large cystic glands in an otherwise normal mucosa. There was also persistence of foci of mucous stasis.

Changes in Other Tissues.—The most uniformly seen change after the first few days was marked ectasia of the veins and lymphatics, and

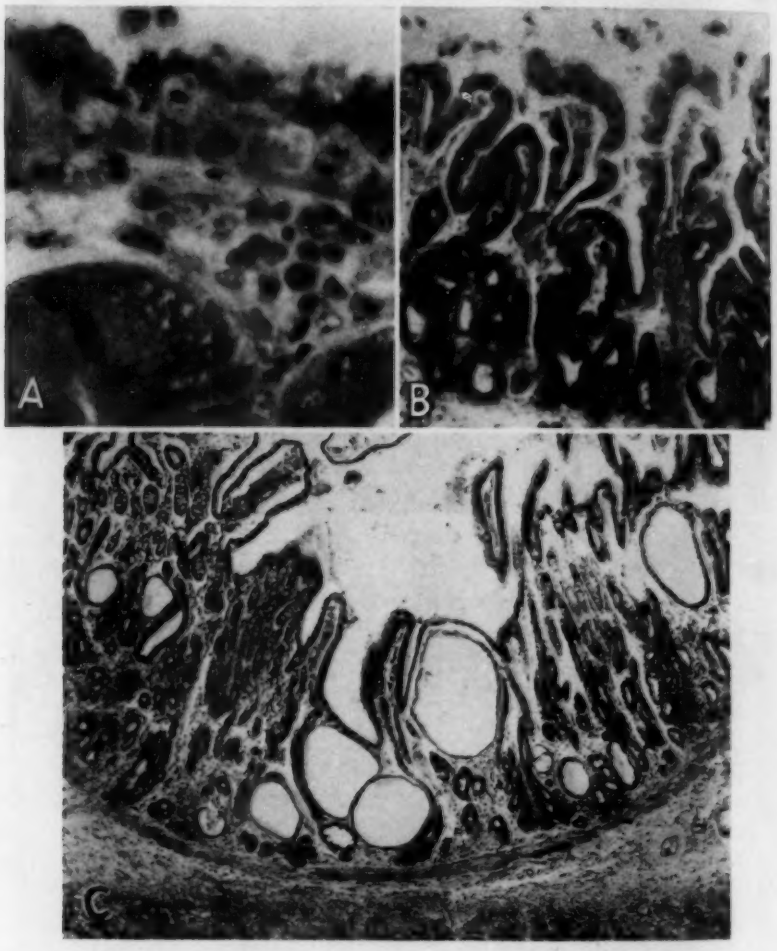


Fig. 2.—*A*, colon first month after irradiation, with miliary necrosis in the surface epithelium and adherent dark masses of bacteria (× 500). *B*, small intestine first month after irradiation, with regenerating glands (× 90). *C*, colon fourth month after irradiation, with cystic glands (× 45). Note slight atrophy and fibrosis of the mucosa.

this was still present after six months. The arteries showed surprisingly little change. Occasionally there were thromboses of vessels in the bases

of advancing ulcers, but in the earlier stages of erosion and ulceration none were seen.

Edema of the connective tissue also was present almost from the outset, but during the third month the connective tissues became hyalinized. By the sixth month the submucosa in particular was markedly thickened and hyalinized. The hyalinization suggested "setting" or gelling of the chronically edematous stroma.

The lymph follicles were rapidly destroyed at the outset and were wiped out by the conclusion of the radiation period. There was,

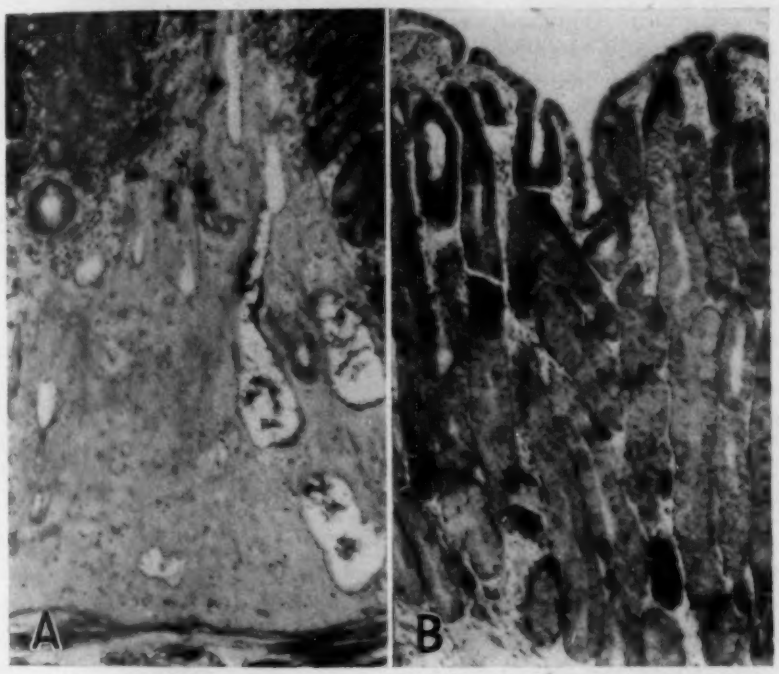


Fig. 3.—*A*, small intestine sixth month after irradiation (× 100). Note thickening and hyalinization of the submucosa with ectasia of veins and lymphatics. *B*, colon first month after irradiation, showing focal stasis of mucus (× 90).

however, persistence of pigment-laden macrophages at the follicle sites. Repair of the lymphoid structures set in after about one month and was concluded several weeks later. There was some failure of formation of normal lymphoepithelial arches in the specialized regions.

Ulceration.—The earliest erosions were present at the end of the period of radiation. They consisted of miliary necroses, usually in altered surface epithelium. There was inflammatory reaction in the underlying stroma, and sometimes bacterial masses were present at the

damaged sites. By the second or third week after the end of the radiation period, extensive erosion and superficial ulceration were frequently seen. The ulcerative lesions became more marked during the second and third months, progressing through the muscle coats, perforating and even forming fistulous tracts when there was extensive necrosis of the bowel wall. Some ulcers were discrete and about 1 cm. in size, while others were more irregular and extensive.

Persistence of altered and atrophic mucosa and of the accompanying regenerative processes was especially marked in the neighborhood of ulcers. In the bases of ulcers were bizarre fibroblastic forms comparable to those seen in the radiation lesions of human patients.[1a] Hyalinization of connective tissue and exudation of fibrin were present but were less marked than in human lesions. The vascular alterations have been described. The inflammatory cellular exudate presented no specific features.

Ulceration was severe and frequent in the colon, while in the small intestine it rarely advanced beyond the stage of erosion. Only one grossly visible ulcer was present in the small intestine in the entire series, though there were many such lesions in the colon.

COMMENT

The data indicate that ulceration developed in a mucosa altered and damaged by radiation. The mucosal regenerative and reparative processes were adequate to maintain the integrity of the lining throughout most of the intestine, but in certain regions ulcers developed despite active but inadequate repair.

Most of the ulcers formed in the colon, where repair was less active and less rapid than in the small intestine. The mucosa in the colon is subject to considerable mechanical trauma and bacterial action. These factors do not lead to ulceration in a normal mucosa, but they may in a damaged mucous membrane.

The pathologic picture of the local lesions was characterized by their situation in zones of altered, atrophic and inflamed mucosa. The atrophy and transformation of the mucous membrane were relatively early but persistent radiation effects. The early stages of erosion and ulceration in these zones have been described. It seems likely, therefore, that the mucosal alterations precede rather than follow the ulcerative processes and that the damaged mucous membrane is specially susceptible to ulcer formation. The mucosal changes at the edges of ulcers are probably not a secondary reaction to the local lesion.

Obliteration of blood vessels was not seen except in the inflamed bases of progressing ulcers. The explanation that localized vascular

occlusion is responsible for the localization of ulcers is not tenable, although such occlusion may contribute to their progression.

A similar conclusion was reached by Engelstad [2] with regard to radiation ulcers in the stomachs of rabbits. He felt that the radiation effect laid the groundwork on which the ulcers later developed. Ivy and his co-workers [3] pointed out that epithelial healing in intestinal radiation ulcers in dogs may have failed because the stromal substrate was damaged and inadequate. In our experiments too there was evidence of damage to the connective tissues of the mucosa and submucosa.

Species differences may be important. Our present observations were made on rabbits. We have failed to produce chronic ulcers in rats with roentgen rays despite marked cellular alterations and acute reactions. It is interesting that Ferguson [4] found poor healing of surgical defects in the stomachs of rabbits as compared with dogs. He attributed this to the constant presence of food in the stomachs.

SUMMARY

The evolution of intestinal radiation ulcers in rabbits was studied by observing the pathologic changes produced in the intestines by a course of roentgen ray treatments. Observations were made during the period of radiation and at regular intervals thereafter for six months.

It was concluded that irradiation of the intestine resulted in an altered and damaged mucous membrane which was especially susceptible to ulceration. Ulceration, though focal, was not localized by vascular occlusion. It seemed rather the chance effect of mechanical trauma and subsequent infection acting on a mucosa and bowel wall structurally and functionally damaged by radiation and imperfectly regenerated.

---

2. Engelstad, R. B.: Strahlentherapie **63**:139, 1935; Am. J. Roentgenol. **40**:243, 1938.

3. Ivy, A. E.; McCarthy, J. E., and Orndoff, B. H.: J. A. M. A. **83**:1977, 1924.

4. Ferguson, A. E.: Am. J. Anat. **42**:403, 1928.

FACTORS INFLUENCING THE DEVELOPMENT AND TIME OF APPEARANCE OF MAMMARY CANCER IN THE RAT IN RESPONSE TO ESTROGEN

CHARLES F. GESCHICKTER, M.D.
AND
ELIZABETH W. BYRNES, M.D.
BALTIMORE

The experimental production of mammary cancer by administration of estrogens (first accomplished in mice by Lacassagne [1] in 1932) indicated that a substance normally produced in the body and possessing physiologic function could lead to cancer. This method has made it possible to reproduce in laboratory animals (mice and rats) mammary cancers which apparently arise under circumstances similar to the natural conditions that bring about human cancer. It is important, therefore, to inquire into the mechanism by which cancer is thus produced and to compare it with the processes in human cancer.

EFFECT OF ESTROGENIC STIMULATION ON THE AGE OF THE MAMMARY GLAND

The primary effect of estrogen on the mammary gland is to accelerate the development of the duct tree and its supporting fibrous tissue. This is accompanied by ripening or enlargement in the mammary epithelium as well as in the cells of the stroma and addition of new elements through multiplication of cells. If accelerated development and ripening are maintained by continuous and intense estrogenic stimulation, the regeneration of tissue which supplies adult cells may ultimately be exhausted. Under these conditions aging and degeneration occur. In such a senescent gland, with further stimulation both benign and malignant tumors form.

Apparently susceptibility to cancer increases with the physiologic age of the breast rather than with the chronologic age of the animal. Repeated experiments have shown that the maximum size of the breast is attained sooner, but cannot be made to exceed by any significant degree that of the normal adult, by estrogenic stimulation. Increasing the

---

This investigation was aided by a grant from the Dr. Simon Baruch Foundation.

Read before the Section on Pathology and Physiology at the Ninety-Second Annual Meeting of the American Medical Association in Cleveland, June 5, 1941.

1. Lacassagne, A.: Compt. rend. Acad. d. sc. **195**:630, 1932.

amount or the duration of the stimulus beyond physiologic limits can therefore only accentuate maturity and aging in the mammary structures. This brings about pathologic changes which may terminate in

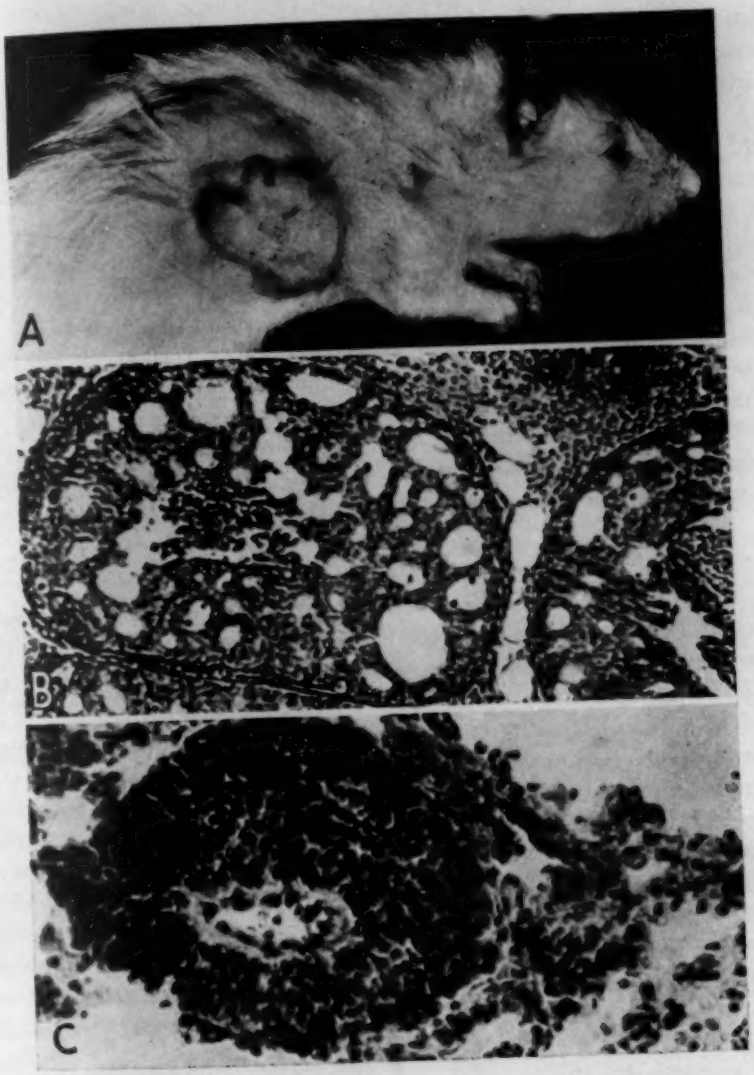


Fig. 1.—Estrogenic mammary cancer: *A*, the appearance of the tumor; *B*, its microscopic structure; *C*, a metastasis to the lung.

cancer. These end stages of estrogenic stimulation occur more quickly if the estrogen is administered in extremely high doses instead of moderately excessive amounts, or if it acts continuously (by dissolution

of implanted pellets)[2] rather than intermittently (by injection of a solution). The essential feature in the estrogenic production of mammary cancer, therefore, is the acceleration or the prolongation of ripening and maturity in the mammary gland beyond physiologic limits. If this condition is satisfied, cancer appears in the breasts of rats which are otherwise immune to the disease, whether these animals are young or old ones, males or females, castrates or noncastrates (fig. 1).

At the present writing, mammary cancers have occurred in 202 of 555 rats of an albino strain which were treated with estrogens. These animals have been maintained on a standard diet and inbred for a period of seven years, and under these conditions no spontaneous mammary cancer has been observed in a colony of over 5,000 animals. The percentage of rats in which estrogenic mammary cancers develop varies, but when the animals survive the required time, the disease may be present in 100 per cent of the survivors under certain experimental conditions. The majority of the experiments herein reported have been carried out in order to learn the time and the mode of onset of the cancers and only a few animals have been allowed to survive with macroscopic growths. Among these, only 2 animals showed multiple metastases to the lungs and lymph nodes.

INTENSITY AND DURATION OF ACTION OF ESTROGENS

A number of estrogenic compounds that vary in potency and in duration of effect may be used for the production of mammary cancer. The potency of each of these compounds and the length of its action are indicated in table 1.

The minimum amount of estrogen necessary to produce a physiologic reaction (cornification of the vaginal smear) in 50 per cent or more of a group of castrated female rats is spoken of as a rat unit and varies from a fraction to several micrograms according to the estrogen and the strain of rats used. If the estrogen has no prolonged action, the rat stays in estrus[3] for two to four days. With prolonged action, estrus may last weeks or months. In the normal rat, estrus usually lasts four to five days, and the amount of estrogen present varies from a fraction to several micrograms. As little as 3 to 5 micrograms of estrone (theelin) administered daily by injection over prolonged periods will produce pathologic changes.

---

2. This statement holds for estrogens if one compares in the same group estrogens without a prolonged effect.

3. The term "estrus" refers to a group of anatomic and physiologic changes in the sexual organs. The uterus is enlarged and its lining thickened. The breasts also increase in size, and the lining of the mammary ducts is thickened. In the vagina, a similar thickening of the mucous membrane is accompanied by the shedding of ripened epithelial cells, and hence the appearance of cornified desquamated cells in the vaginal smear is taken as an index of estrus.

In woman estrus is roughly the time between two menstrual periods. Shorr and Cohen,[4] from observations on castrate women, estimated that from 3,000 to 5,000 micrograms of estradiol benzoate a day is necessary to produce human estrus. In woman, therefore, estrus lasts four to five times as long as in the rat and requires five thousand to ten thousand times as much hormone daily.

In considering the mechanism of mammary cancer it is important to bear in mind the ratios between physiologic and pathologic doses of estrogen and the ratio between the dosage for rats and that for man. As will be shown subsequently, mammary cancer results in rats within a period of one hundred and twenty days (which is approximately thirty times the length of normal estrus) when the amount of estradiol benzoate

TABLE 1.—*Potency and Period of Action of Various Estrogenic Compounds*

Compound	Potency in Millions of Rat Units per Gm. *	Potency in Millions of Rat Units per Gm. †	Authors' Assays	Prolonged Action
Estradiol (alpha)	12	0.5	3.3	No
Estradiol (benzoate)	6	...	1.7	Yes
Estradiol (dipropionate)	5	...	2.5	Yes
Estrone (theelin)	1	0.3	1.0	No
Stilbestrol	3	0.6	3.3	No
Stilbestrol monomethyl ether	..	...	0.4	Yes
Stilbestrol dimethyl ether	..	...	0.04	Yes

* The figures in this column were obtained from the following sources: those for estradiol (alpha benzoate) and estrone from B. Whitman, O. Wintersteiner and E. Schwenk (J. Biol. Chem. **118**: 789, 1937); those for estradiol dipropionate from K. Miescher, C. Scholz and E. Tschopp (Biochem. J. **32**: 725, 1938); those for stilbestrol from E. C. Dodds, L. Golberg, W. Lawson and R. Robinson (Nature, London **142**: 34, 1938).

† These figures are from C. W. Sondern and J. L. Sealey (Endocrinology **27**: 670, 1940).

administered daily is approximately ten to fifteen times the physiologic dose. If the same factors are applied to the human breast, the length of time would approximate ninety weeks and the daily dose of the hormone would approximate 30 to 75 mg. This assumption has only theoretic interest since there is at present no way to evaluate the matter of species differences. Thus in experiments carried out by us on rabbits and monkeys cancers have failed to appear when corresponding amounts and periods of estrogenic stimulation were employed.[4a] Moreover, the calculated dose for man is based on the assumption that the subject is young or in the period of sexual maturity and that all of the organs are

4. Shorr, E., and Cohen, E. J.: Proc. Soc. Exper. Biol. & Med. **46**:330, 1941.

4a. Mammary cancer recently appeared in 1 rabbit after twenty months (six hundred and fifteen days) of treatment with stilbestrol dimethyl ether (in the dosage of 0.5 mg. daily). Cancers have failed to develop to date in a group of 6 monkeys stimulated continuously for more than three years with estrone and estradiol compounds implanted as pellets or injected (the total dose of estrogen exceeding 3 Gm. per monkey).

in their normal state. There are conditions (as will be pointed out subsequently) in which the cancer-producing threshold is greatly lowered.

There are two proofs that estrogens exert physiologic rather than direct chemical action in producing mammary cancer. One is that the cancer does not occur at the site of injection but appears, instead, in the organ (the breast) which the hormone influences physiologically. The second is that the periods of time required to produce cancer with estrogens of varying chemical composition are proportional to the physiologic potencies and independent of the chemical formulas (table 2 B and D). Therefore the estrogens cannot be considered chemically as cancerogenic agents.

Cancer occurs in the rat's breast when the estrogenic stimulation is (*a*) abnormally intensified, (*b*) prolonged and continuous or (*c*) augmented by other influences, such as previous endocrine disturbances. Intense interrupted stimulation is not as efficient as continuous estrogenic action obtained by the implantation of pellets or by the use of estrogens with prolonged action.

Relation of Intensity of Stimulation to Time of Appearance of Mammary Cancer.—The total amount of estrogen necessary to produce cancer is the same regardless of the daily dose administered if animals of the same age are treated by injection of estrone in oil. Thus it can be shown that with daily injection of estrone in oil the time required to produce mammary cancer in the rat is inversely proportional to the dose but that the total amount remains fairly constant (30 to 40 mg. of estrone). This was found true if daily doses of 50 to 200 micrograms were injected. It is possible that with daily doses beyond 200 micrograms of estrone increased excretion or destruction would occur and the time required for the appearance of the cancer would not be lessened.

Mammary cancer results when estrone in oil is injected subcutaneously in the backs of rats in daily doses above 0.025 mg. (25 micrograms). The cancers affect males and females, castrates and noncastrates. The cancers appear in five hundred to six hundred days when 50 micrograms is injected daily, within three hundred and fifty to four hundred days with 100 micrograms and within one hundred and fifty to two hundred days with 200 micrograms (table 2 A, B and C).

The relation of the intensity of stimulation to the time of appearance of mammary cancer may be demonstrated in another way by comparing the periods of time required to produce cancers with estrogens of varying potency. The time required varies in accordance with the physiologic strength of the compounds. The most potent estrogen will produce cancer in the briefest time [2] (table 2 D).

Effect of Interrupted Doses (Daily Injections of Estrone in Oil).—Because of the rapid ripening of the lobular buds when large amounts of estrogen are injected daily, immense dilated acinar structures or cysts

TABLE 2.—*Incidence and Time of Occurrence of Mammary Cancers in Rats Receiving Varying Doses of Estrogen in Oil*

Rat	Sex	Age	Castrated	Days Treated	Results
	A. 50 Micrograms of Estrone Daily (3 Cancers at 550-598 Days)				
1	F	1 mo.	No	284	Cystic changes
2	M	1 mo.	Yes	550	Cancer
3	M	1 mo.	Yes	591	Cancer
4	M	1 mo.	Yes	598	Cancer
	B. 100 Micrograms of Estrone Daily (4 Cancers at 359-408 Days)				
1	M	1 mo.	Yes	29	Cystic change
2	F	1 mo.	Yes	37	Cystic change
3	M	1 mo.	Yes	359	Cancer
4	F	1 mo.	Yes	378	Cancer
5	M	1 mo.	Yes	387	Cancer
6	M	1 mo.	Yes	408	Cancer
	C. 200 Micrograms of Estrone Daily (8 Cancers at 137-281 Days and 1 Cancer at 334 Days)				
1	F	1 mo.	Yes	174	Cystic change
2	F	1 mo.	Yes	188	Cancer
3	F	1 mo.	Yes	150	Cancer
4	F	1 mo.	Yes	334	Cancer
5	M	1 mo.	No	300	Cystic change
6	M	1 mo.	No	300	Atrophy
7	M	1 mo.	No	281	Papillary cancer
8	M	1 mo.	No	300	Cystic change
9	M	1 mo.	No	194	Duct cancer
10	M	1 mo.	No	300	Cystic change
11	F	1 mo.	No	234	Atrophy
12	F	1 mo.	No	260	Cystic change
13	F	1 mo.	No	137	Duct cancer
14	F	1 mo.	No	197	Cystic change
15	F	1 mo.	No	224	Lobular and duct cancer
16	F	1 mo.	No	238	Duct cancer
17	F	1 mo.	No	224	Cystic change
	D. 100 Micrograms of Stilbestrol Daily (8 Cancers at 104-222 Days. Compare with B)				
1	M	2 mo.	No	92	Cysts, fibrosis and atrophy
2	F	2 mo.	Yes	104*	Cancer
3	F	2 mo.	Yes	163	Sweat gland adenoma
4	F	2 mo.	Yes	202	Cancer
5	M	2 mo.	No	222	Cancer
6	F	1½ mo.	No	130	Duct cancer; very early comedo cancer
7	F	1½ mo.	No	130	Small comedo duct cancer
8	F	1½ mo.	No	130	Duct cancer
9	F	1½ mo.	No	130	Very early comedo cancer
10	F	1½ mo.	No	130	Very early lobular cancer

* This rat received 200 micrograms daily.

are formed. These cysts appear within twenty to forty days and are accompanied by secretory changes in the epithelium. The cysts are preceded by increase in the size and the number of the epithelial buds at the ends of the tubules. The cysts have no direct relation to the subsequent formation of cancer and are associated with rapid ripening and secretion in the epithelium of the terminal tubules (fig. 2*A*). When cysts form,

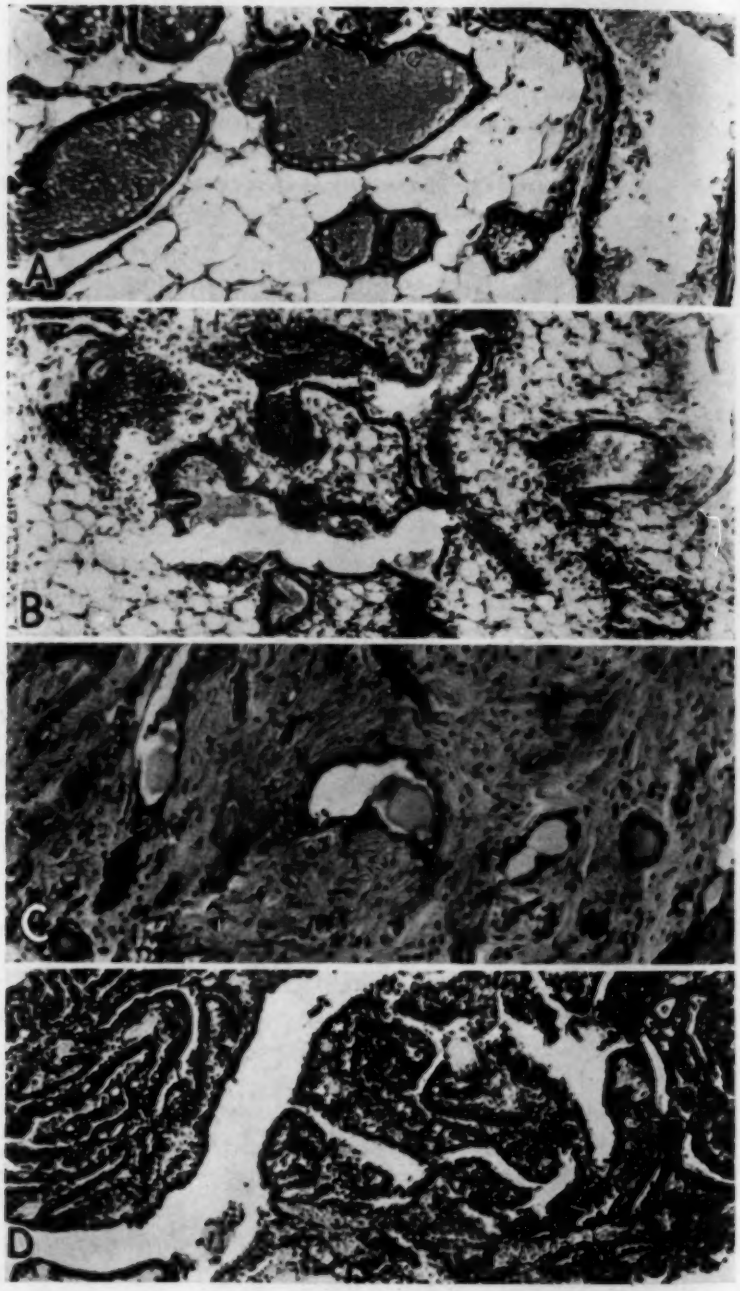


Fig. 2.—Pathologic changes in the mammary gland preceding the development of estrogenic mammary cancer: *A*, large cysts occurring after daily injections of estrone in oil; *B*, adenosis and epithelial hyperplasia following implantation of pellets; *C*, fibroadenoma following implantation of pellets; *D*, benign papilloma following implantation of pellets.

the appearance of the cancer is delayed, and a more prolonged estrogenic effect with continuous epithelial regeneration followed by degeneration and atrophy must occur before cancer appears. This corresponds with

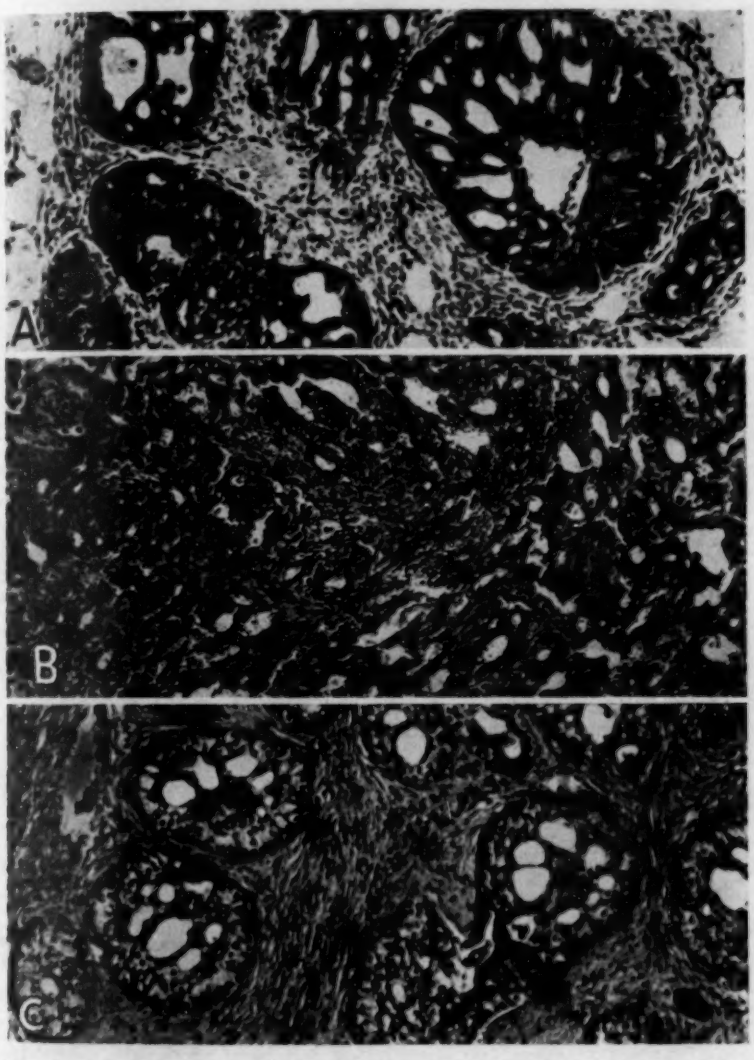


Fig. 3.—Varying forms of mammary carcinoma with different methods of dosage: *A*, comedo cancer obtained with interrupted daily doses; *B*, lobular cancer obtained by implantation of pellets; *C*, duct cancer obtained by implantation of pellets.

observations in the human breast which indicate that cystic disease occurring in women toward the menopause is not associated (except

coincidentally) with mammary cancer. It is usually a self-limited disease and associated with a relatively brief period of estrogenic overstimulation at or near the menopause.

With intense interrupted estrogenic stimulation (daily injections of estrone in oil) in castrate female rats, lobule formation does not occur in the breast, and as a result the cancers formed are confined to the ducts and terminal tubules, and their pathologic variety is thus limited. Under these conditions comedo cancer is the predominant pathologic form (fig. 3).

Effect of Continuous Absorption (Implantation of Pellets).—When an estrogen is injected in oil, absorption is rapid for the first several hours, but soon thereafter the concentration in the tissues diminishes. Under these conditions there are daily variations in the estrogenic level. The agent is more effective if the concentration is maintained by more gradual and constant absorption, as from pellets [5] implanted beneath the skin of the back.

Mammary cancer in the rat occurs with smaller amounts of estrogen when pellets of the agent are implanted (tables 3 and 4). As little as 9 to 10 mg. of estrone in the form of two to three pellets given individually at intervals will produce cancer in two hundred and fifty to three hundred days. Although when pellets are used mammary cancers are produced with less estrogen than when injections are used, not all the animals show the same rate of development of cancer, and cancer does not appear in all of them. In young animals benign tumors (papilloma or fibroadenoma) may first appear (fig. 2). Later, cancer may occur in the benign fibroadenoma, after the latter has undergone involutional changes. When estrone pellets are used in castrated male rats, most of them die within two to three months from urinary obstruction caused by epidermal thickening in the urethra.

The formation of fibroadenoma in response to estrone from pellets is due to the selective action of moderate, continuous and prolonged estrogenic stimulation on the connective tissue. The fibrous stroma of the breast is apparently more susceptible to such moderate and prolonged stimulation than to the short intense stimulation of high doses. The fibrosis occurring in the mammary gland, therefore, serves as an index of the duration and the intensity of the estrogenic stimulation. When it is most marked, the stimulation has been moderate, continuous and of long duration.

5. These pellets are small plugs of crystalline estrogen, weighing between 2.5 and 3.0 mg. They are made by tightly compacting the crystals in drilled holes in a steel plate. The compactness of the pellet is such that absorption takes place only from the surface, slowly and continuously.

If the ovaries are present when pellets are implanted, the slow continuous absorption of the estrogen results in more prolonged and increased luteinization of the ovaries and in mammary lobule formation approaching that seen in the breast in pregnancy. Even in the absence of the ovaries estrone from the pellets stimulates moderate, irregular lobule formation. The lobulation is significant, since it influences the

TABLE 3.—*Mammary Cancers Produced in Rats by Implantation of Estrone Pellets Weighing 2.5 mg. Each*

Rats	Sex	Total Dose, Mg.	Day of Life on Which Pellets Were Implanted	Tumors Produced	Days Required to Produce Cancer
7	F	5.5 †	2d 28th 153d	Cancer * in 4; benign fibroadenoma only in 2 (135 and 190 days) .	255 to 350 days *
5	M	5.5	2d 28th 153d	Cancers in 2; fibroadenoma in 0	172 to 300 days

* One animal with four small pellets implanted on the twenty-third day of life had microscopic cancer on the forty-fourth day of life; other microscopic cancers appeared from one hundred and seventy-two to three hundred and fifty days after the starting of treatment. In 2 of the 4 females in which cancer developed, benign fibroadenoma of the breast developed preceding cancer. In 2 additional females, benign fibroadenoma only developed.

† Since the pellets were not completely absorbed, the dose was less than the total weight of the pellets (7.5 mg.). These rats were not castrated.

TABLE 4.—*Early Cancer with Multiple Pellets—Total Dose 12.5 mg.*

Rat	Age	Sex	Castrated	Estrogen	Days Required for Cancer
1	21 days	F	Yes	Estrone	21
2	26 days	F	Yes	Estrone	25
3	21 days	F	Yes	Estrone	30
4	21 mo.	F	No	Estrone	47
5	21 mo.	F	No	Estrone	60
6	21 mo.	F	No	Estradiol	47
7	60 days	F	Yes	Estrone	60
	15 mo.	F	No	Stilbestrol	35
8	9 mo.	F	Yes	Stilbestrol	90

pathologic form of the mammary cancer. Under these conditions infiltrating lobular cancers may form (fig. 3).

The more gradual and continuous absorption of an estrogen from implanted pellets results in a more widespread and extensive epithelial response. Epithelial proliferation may for a period of months predominate over epithelial ripening and secretion. Adenosis with formation of duct adenoma and intraductal papilloma occurs in addition to fibroadenoma and precedes or replaces the formation of large cysts

(fig. 2). Again the epithelial changes are not in themselves precancerous. This is proved by their gradual involution and disappearance if the pellets are removed (or by the absence of cancer if the initial low concentration of estrogen is maintained by daily injection of physiologic amounts of the agent throughout the life of the animal in the absence of pellets). However, once these epithelial changes have been produced, the mammary gland is more susceptible to cancer *provided a steadily increasing concentration of estrogen is maintained* by allowing the pellets to remain and by implanting additional ones.

The modified action of estrogen administered by implantation of pellets instead of by injection is also apparent when stilbestrol or estradiol is administered. With daily injections of 100 micrograms

TABLE 5.—*Comparison of Doses and Periods of Treatment Required with Different Estrogenic Compounds*

Compound	Dose, Micrograms	Total Amount, Mg.	Days Required to Produce Cancer
Estradiol (injected)	100	25	290
Estradiol (implanted)	3 successive pellets	8	310
Estradiol dipropionate	5	0.6	120
Estradiol benzoate	5	0.6	120
Stilbestrol (injected)	100	15.0	150
Stilbestrol (implanted)	5 successive pellets	10.1	105
Stilbestrol monomethyl ether	50	9.5	212
Stilbestrol dimethyl ether *	500	116.5	233

* The cancer-producing efficiency of estrogen derivatives with a prolonged action is not apparent when the estrogenic potency of the derivative is far below that of the parent compound (table 1).

of stilbestrol, 18 mg. of the substance is necessary for the production of mammary cancer, whereas only 10 mg. is required if pellets are used. With daily injections of 100 micrograms of estradiol in oil, 25 mg. is required to produce cancer; with implantation of pellets 8 mg. will suffice. The more continuous estrogenic action observed with stilbestrol and estradiol pellets is associated with the formation of fibroadenoma, adenosis and other changes similar to those described in the animals receiving estrone pellets. In general, the total amount of the estrogen necessary to produce cancer may be decreased to one-half or to one-third if the agent is implanted in the form of pellets rather than injected in oil (table 5).

Instead of estrone, estradiol or stilbestrol pellets, estrogenic compounds with a prolonged action may be used. The tissue changes are similar to those produced by the implantation of pellets.

Estradiol compounds in which a chemical radical is introduced to prolong estrogenic action are more efficient in producing cancer than the original compound even though the estrogenic potency has been lessened somewhat by the addition of the radical. This is also true of the ethers of stilbestrol which have a prolonged action.

Table 5 gives a comparison of the amount of the agent and the time required for production of cancer with derivatives of estradiol injected in oil as compared with the original compound administered by injection or in the form of pellets. Table 5 also gives the corresponding information for stilbestrol and its derivatives.

Spontaneous mammary cancer is rare in the rat and is difficult to maintain by transplantation. Estrogenic mammary cancer in the rat, therefore, provides valuable experimental material for the study of the

TABLE 6.—*Percentage of Rats in Which Mammary Cancer Developed When Varying Amounts of Estradiol Benzoate in Oil Were Injected Daily*

Rats *	Age at Onset of Treatment	Dose, Micrograms	Duration of Treatment	Cancers	Percentage
5	2 mo.	5	120 days	5	100
5	2 mo.	10	120 days	5	100
12	7 mo.	50	120 days	6	50
12	1½ mo.	100	120 days	9	75
8	6 mo.	100	120 days	5	62
22	7-9 mo.	100	120 days	11 †	50
6	1½ mo.	200	120 days	4	67
70				45	

* The numbers include both males and females; none of the rats were castrated.
† The majority of these cancers occurred in eighty to one hundred days.

disease. These cancers can be produced at an early age and relatively quickly if the proper estrogen and mode of administration are used. In our experience, cancers are produced most quickly and in a high percentage of the animals if 5 micrograms of estradiol benzoate is injected daily except Sundays in rats from 1 to 3 months of age. With this dose, the tumors appear in microscopic form in one hundred and twenty days, and palpable nodules appear shortly thereafter in one hundred and twenty-five to one hundred and seventy days. When more than 5 micrograms of estradiol benzoate is given daily, the cancers appear at the same rate, and the percentage of rats in which cancer develops is not increased but tends to decrease.[6]

6. With the daily injection of 100 micrograms of stilbestrol in oil, cancer appeared in approximately 100 per cent of the rats in one hundred and forty to one hundred and fifty days.

EFFECT OF PROLONGED REGENERATION

Since the susceptibility to cancer is influenced by the capacity for regeneration in the tissue the preexisting physiologic state of the gland is important. If epithelial regeneration below the cancer-producing level has been previously stimulated in the mammary gland, it should be possible at some later date to produce cancer more readily.

In a group of rats 1 month old, intense estrogenic stimulation (200 micrograms of estrone) was given for ninety days. Biopsy showed that no cancers had developed. These rats were allowed to live one and a half years without further treatment, and at the end of this time short intense estrogenic stimulation was given. Cancer appeared quickly in from two to three months. In another group of rats physiologic doses of estrone (2.5 to 5 micrograms) were given daily for a period of almost two years, and no cancers appeared. In these animals at the end of this time cancer developed within two months after the injection of large daily doses of estrone. Thus, the effects of a previously intense or prolonged period of estrogenic stimulation may persist as a latent cancerogenic influence in the breast (tables 7 and 8).

Because of the latent predisposition to cancer in the breasts of these animals, the appearance of these glands under the microscope before the final period of estrogenic stimulation is important. In the group that had intense stimulation for ninety days in early life, the glands were markedly atrophic and nearly replaced by fat. Their structure resembled the so-called "senile, fatty breast" so often described in pathologic reports on human breasts removed for cancer.

In the group of rats which had been castrated and which received daily injections of estrone in doses within physiologic limits (2.5 to 5 micrograms), the mammary glands were markedly fibrosed and the terminal tubules showed irregular epithelial proliferation resembling adenosis or Schimmelbusch's disease in the human breast (fig. 2 *B*). It is significant that women with adenosis when observed at intervals through a period of years (and past the menopause) show a greater tendency toward mammary cancer than those with normal breasts in the same age groups.

It is in these last two experiments in which preexisting changes in the breast (resulting from previous estrogenic stimulation) combine with the effects of a final period of intense estrogenic action to produce cancer that conditions found in the human breast with cancer are most closely approximated. These experiments suggest that human mammary cancer may result from one or a combination of the following factors:

1. Abnormally intense estrogenic stimulation during the adolescent period of mammary development or during a previous pregnancy.

(Some of these patients will recall varying degrees of virginal or gravid hypertrophy.)

2. Ovarian dysfunction in cyclic women resulting in relative hyperestrogenism over a period of years prior to the menopause. (Most of

TABLE 7.—*Cancer Produced in Castrated Rats by Administration of Initial Massive Doses of Estrone at the Age of 1 Month Followed by an Interval of No Treatment, Then by Further Administration of Massive Doses*

Sex of Rat Used	Initial Dose		Interval Without Treatment, Days	Final Dose		Microscopic Picture	
	Amount, Micrograms	Duration, Days		Amount, Micrograms	Duration, Days	Days After Initial Dose	Days After Final Dose
F	200	90	563	2 pellets into breast	80	338, fibrosis 522, infected nipple 620, atrophy 652, atrophy	23, hyperplasia 89, cancer
M	200	90	563	2 pellets into breast	54	144, fibrosis 565, fibrosis, irregular lobules 652, atrophy	23, hyperplasia 54, cancer
M	200	90	563	2 pellets into breast	54	565, fibrosis 652, atrophy	23, hyperplasia 54, cancer
F	200	90	754	None, control	..	216, fibrosis 620, fibrosis 675, atrophy 741, senile atrophy	90, no change
M	200	90	...	None, control	..	620, atrophy 675, atrophy	90, no change

TABLE 8.—*Cancer Produced in Castrated Rats by Administration of Large Doses of Estrone Following Prolonged Administration of Physiologic Doses Begun at the Age of 1 Month*

Sex of Rat Used	Physiologic Dose		Massive Dose		Microscopic Picture	
	Amount, Micrograms	Duration, Days	Amount, Micrograms	Duration, Days	594 Days After Physiologic Dose	Days After Massive Dose
M	2.5	594	200	98	Irregular lobules and moderate fibrosis	10, negative for cancer
M	2.5	594	200	98	Cystic dilatation, some adenomatous lobules	23, fibroadenoma 41, precancerous changes 98, comedo cancer
F	5.0	594	200 Pellet, 3 mg. 200	11 13 74 98	Cysts and lactating adenoma	11, atrophy 23, comedo cancer 41, duct cancer 68, gross cancer 98, large cancer and fibroadenoma
F	5.0	594	200 Pellet, 3 mg. 200	11 13 74 98	Fibrosis and fibroadenoma	11, fibroadenoma 41, comedo cancer 98, gross cancer

these patients have the characteristic changes of adenosis or Schimmelbusch's disease.)

3. Intense or continuous estrogenic stimulation occurring at the time of menopause (superimposed on the factors enumerated under 1 and 2).

EFFECT OF AGE

According to vital statistics, the incidence of human mammary cancer increases steadily with the age of the population. The atrophy and the degenerative changes found in the senile breast may therefore be etiologic factors in mammary cancer. With respect to estrogenic mammary cancer in the rat, histologic studies indicate that premature ripening and accelerated regeneration which terminate in degenerative changes may be precursors of cancer. In line with this interpretation a comparison was made of the periods required for the appearance of cancer in young and old rats.

It was found that the time required for the appearance of cancer was inversely proportional to the age of the animal in a series in which

TABLE 9.—*Time Required to Produce Mammary Cancer with Multiple Estrone Pellets in Rats of Varying Ages*

Rats	Age	Sex	Number of Pellets Administered	Cancers	Time Required, Days	Average Number of Days
30	1 wk.	F	One at a time	17	105-532	300
10	1 mo.	Both*	Multiple	4 2	175-385 21-25	293
10	2½ mo.	F	Multiple	5 1	175-317 26	248
10	4 mo.	Both	Multiple	6 1	101-399 35	248
6	7½ mo.	Both	Multiple	3 1	165-255 31	205
4	9 mo.	F	Multiple	2	180-210	195
8	20 mo.	F	Multiple	4 2	64-109 27-48	90

* The group was made up of males and females in equal numbers. Cancer developed in both sexes with equal frequency.

estrone pellets were implanted, but there were a certain number of exceptions in which cancers appeared early regardless of the age (table 9). With other methods of estrogenic stimulation, no significant difference between young and mature rats was found. Thus, as shown in table 10, the majority of the mammary cancers were observed within one hundred and twenty days regardless of whether the injections (100 micrograms of estradiol benzoate) were begun at the age of 1½, 6 or 11 months. A certain number of early cancers occurred in all of the experimental animals in which continuous absorption of the estrogen took place, particularly if multiple pellets were inserted. These early cancers developed within twenty-one to ninety days after treatment and were dependent on the method of dosage and were independent of the age of the animals (table 4). When the data were summarized for all methods of estrogenic stimulation, the chronologic age was found to be

without significance for the time of appearance of the mammary cancers. Additional experiments, utilizing rats aged 20 months or more, are being carried out since an insufficient number at this age have been tested.

EFFECT OF OTHER SEX HORMONES

Injections of either progesterone or testosterone over a long period alone will cause increased lobular development in the mammary gland of the rat whether the animal is castrate or noncastrate, male or female.

TABLE 10.—*Results with Administration of 100 Micrograms of Estradiol Benzoate*

Rats Used	Age of Rats When Treatment Started	Results of Biopsy		Cancers at Autopsy After 120 Days of Treatment
		After 78 Days of Treatment	After 105 Days of Treatment	
6 Females	1½ mo.	.....	1 cancer	5
6 Males	1½ mo.	2 cancers	.....	4
4 Females	6 mo.	.....	Fibroadenoma only	2
4 Males	6 mo.	.....	Adenosis and cysts only	3
2 Females	11 mo.	.....	Adenosis only	1

TABLE 11.—*Mammary Cancer in Rats Receiving Testosterone and Estrone*

Rat Used	Dosage of Testosterone	Dosage of Estrone	Result
Female castrate	0.5 mg. 1-18 days	100 micrograms, 30-57 days 200 micrograms, 57-162 days	Autopsy after 162 days: cancer and adenosis
Female castrate	0.5 mg., 73-108 days 0.5 mg., 143-180 days	200 micrograms, 31-72 days 200 micrograms, 108-143 days	Autopsy after 192 days: comedo cancer
Male castrate	0.5 mg., 285-357 days	25 micrograms, 25-285 days 50 micrograms, 357-407 days Estrone pellet, 407th day, 493d day	Autopsy after 530 days: cancer, perithelioma-tous type
Female castrate	0.115 mg., 80-249 days 1.25 mg., 249-266 days	10 micrograms, 80-249 days	Comedo cancer after 266 days of treatment
Female castrate	0.25 mg., 79-249 days	20 micrograms, 79-249 days	Duct cancer after 99 days of treatment
Male intact	1 pellet	5 pellets	Cancer after 383 days
Female intact	0.5 mg., 43-73 days*	5 pellets	Comedo and lobular cancer after 73 days

* Progesterone was substituted.

Testosterone is more efficacious than progesterone in producing lobular growth. No cancers were observed in the breasts when injections of progesterone alone were given over a period of several months or when testosterone was given over a period of more than a year; the only significant mammary change was a lobular formation resembling that of midpregnancy. However, pathologic changes and cancer formation occurred if injections of testosterone or progesterone were given simultaneously with injections of estrone or when a similar combination of pellets was given (table 11). Under such endocrine stimulation the formation of large mammary cysts was prevented. The degenerative changes preceding cancer appeared in the acinous structures and

resembled postlactation involution. The earlier changes resulting from combined hormone stimulation were characterized by irregular epithelial proliferation resembling that seen in the human breast with adenosis. It is significant that the addition of testosterone or of progesterone did not prevent the occurrence of mammary cancer in these experiments. Instead, in some animals lobular cancers were apparently the result of stimulation by these hormones, since the amount of estrogen used was insufficient by itself to produce the cancers. The gonad-stimulating and the lactogenic hormones of the anterior lobe of the pituitary gland which produce lobule formation and secretion in the mammary gland of intact animals will, however, inhibit the growth of estrogenic mammary cancer. This is discussed subsequently.

CHANGES IN THE ENDOCRINE ORGANS IN THE EXPERIMENTAL PRODUCTION OF MAMMARY CANCER

Pathologic changes in the endocrine organs have been reported by most of the workers who have produced mammary cancer with high doses of estrogens in mice (Cramer and Horning [7]). The pituitary gland enlarges because of an increase in the number of chromophobe cells in its anterior lobe. Such changes were found at autopsy in the animals in the present experiments (fig. 4). In addition these animals showed degenerative changes in the gonads with formation of benign cysts or papillomatous cysts of the ovary (2 animals had granulosa cell cancer, another metastasizing adenocarcinoma and another mesonephroma of the ovary). There was enlargement of the adrenal cortex, and occasionally there were degenerative changes in the medulla. Cystic degeneration occurs in the thymus gland with compensatory hypertrophy of the mediastinal lymph nodes, which may terminate in lymphosarcoma or leukemia (table 12).

The most striking and consistent changes are in the pituitary gland and in the thymus. The increase in the number of chromophobe cells in the anterior lobe of the pituitary leads to hypertrophy of adenomatous proportions. Most of the experimental animals have glands five or more times normal size, and hemorrhage may occur in the adenomatous tissue (fig. 4). The significance of these changes in the development of mammary cancer is not established. Hypophysectomy prevents the physiologic effects of estrogen on the mammary gland, and no cancers develop. The importance of the pituitary gland for the development and normal physiologic response of the breast has been stressed by other workers (Gomez and Turner [8]). It has also been noted in the present

7. Cramer, W., and Horning, E. S.: Lancet **1**:247, 1936.

8. Gomez, E. T., and Turner, E. W.: Hypophysectomy and Replacement Therapy in Relation to the Growth and Secretory Activity of the Mammary Gland, Research Bulletin 259, University of Missouri College of Agriculture, Agricultural Experiment Station, 1937.

group of experiments. Estrogen response was prevented by hypophysectomy (Astwood, Geschickter, and Rausch [9]). Hypophysectomy prevented the appearance of cancer even in those animals which lived the required length of time (six months or more) and in which the removal of the pituitary was apparently incomplete.[10]

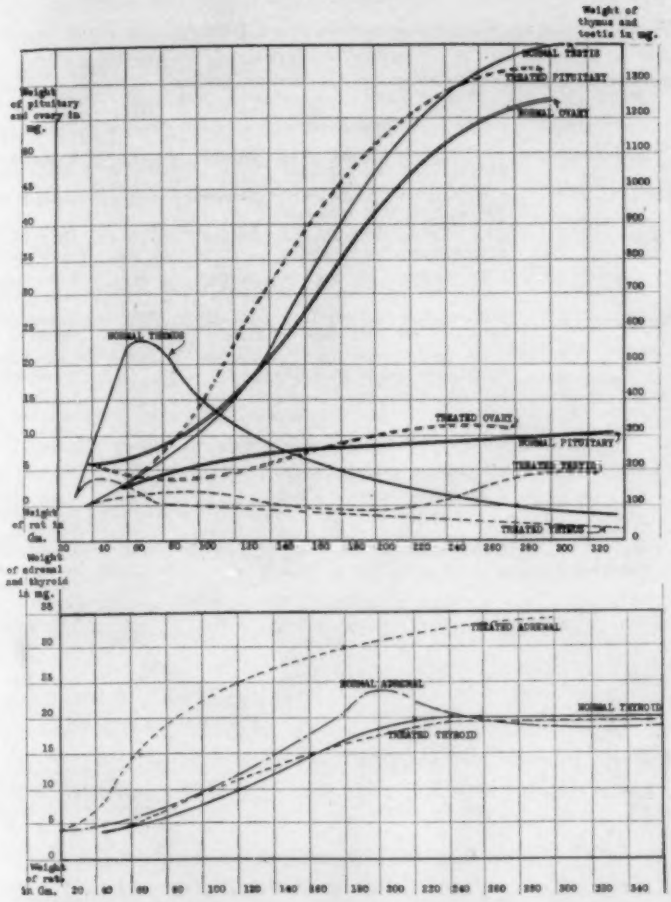


Fig. 4.—Effect of developing estrogenic mammary cancer on the endocrine glands of rats: Upper graph, effect of prolonged administration of estrogens on the weights of the pituitary, the thymus and the gonads; lower graph, effect on the weights of the adrenal and the thyroid gland.

9. Astwood, E. B.; Geschickter, C. F., and Rausch, E. O.: Am. J. Anat. **61**:373, 1937.

10. The hypophysectomized animals used in the experiments were prepared by Dr. R. O. Greep of the Squibb Institute for Medical Research.

Striking involutional changes occur in the thymus glands of rats maintained under continuous overstimulation with estrogen. The glands shrink in size, and the thymic tissue is replaced by cysts lined with transitional epithelium resembling that seen during the embryologic development of the thymus and the thyroid. This thymic involution is

TABLE 12.—*Lymphosarcoma and Myeloid Leukemia in Rats Treated with Estrogens*

Rat	Age	Sex	Treatment	Days Treated	Results
1	1 mo.	F	2 hr. radium (30 mg.) over thymus; 100 mg. estradiol benzoate	225	Lymphosarcoma; cancer of breast
2	1½ mo.	F	2 hr. radium (30 mg.) over thymus; 100 mg. estradiol benzoate	220	Lymphosarcoma
3	1½ mo.	F	2 hr. radium (30 mg.) over thymus; 100 mg. estradiol benzoate	210	Lymphosarcoma; cancer of breast
4	3½ mo.	F	5 hr. radium (30 mg.) to mediastinum; 100 mg. estradiol benzoate	145	Lymphosarcoma; cancer of breast
5	3½ mo.	F	6 hr. radium (30 mg.) to mediastinum; 100 mg. estradiol benzoate	165	Lymphosarcoma; cancer of breast
6	4 mo.	F	6 hr. radium (30 mg.) to mediastinum; 100 mg. estradiol benzoate	150	Lymphosarcoma; cancer of breast
7	20 mo.	F	100 mg. estradiol benzoate daily	600	Lymphosarcoma of spleen; cancer in fibroadenoma of breast
8	1 wk.	M	3 successive estrone pellets.....	150	Myeloid leukemia
9	1 wk.	F	3 successive estrone pellets.....	420	Cancer of breast; lymphosarcoma of thymus
10	1 wk.	F	3 successive estrone pellets.....	420	Lymphosarcoma of thymus and spleen; cancer of breast
11	1 wk.	F	3 successive estrone pellets.....	480	Lymphosarcoma of spleen; cancer of breast
12	1 wk.	F	3 successive estrone pellets.....	300	Lymphosarcoma of thymus and mesenteric nodes; cancer of breast
13	1 wk.	M	3 successive estrone pellets.....	420	Lymphosarcoma of thymus; cancer of breast
14	1 mo.	M	5 estrone pellets.....	300	Lymphosarcoma of breast; beginning cancer of breast
15	13 mo.	F	6 stilbestrol pellets—repeated in four months	165	Lymphosarcoma; cancer of breast
16	21 mo.	M	5 estrone pellets.....	60	Lymphosarcoma

marked after prolonged administration of estrogen but also occurs when other sterol hormones, e. g., testosterone and progesterone, are administered. Whether or not it plays a role in the development of mammary cancer is being investigated further.

Changes in the adrenal glands are most frequently found in the reticular zone of the cortical tissue. The size of the cells and their lipid contents are increased. The result is a moderate increase in the size and

the weight of these glands in the animals receiving estrogens and in those in which mammary cancer develops. The changes in the adrenal medulla are variable. Degeneration and calcification are occasionally seen, but most often the appearance of this tissue is normal.

No significant changes were found in the thyroid glands of the treated animals (fig. 4 *B*), although marked changes with squamous cell metaplasia have been reported in response to estrogens by other workers (Mark and Biskind [11]).

The atrophic changes in the gonads are probably significant in the development of mammary cancer. There were no apparent differences in percentage of cancers or in time of their appearance between castrate and noncastrate rats whether these were males or females, but this may be explained by the atrophy of the gonads when estrogen is administered in large amounts.

The size of the ovaries may be reduced from a diameter of 5 to 8 mm. in the normal adult to a millimeter or less in those animals which have had prolonged treatment with estrogen. In the atrophic ovaries, there is a decrease in the number of follicles, and those persisting are decreased in size or undergo cystic degeneration. Luteal changes are absent. The interstitial cells are increased in number but are small and compact. Cysts with intracystic papillomas are occasionally observed. In 4 animals malignant ovarian tumors occurred. One of these was a metastasizing adenocarcinoma; another was a small and malignant mesonephroma (fig. 5). In the animal in which adenocarcinoma of the ovary developed, squamous cell cancer of the cervix was also present, as well as mammary carcinoma. This was the only animal with a definitely malignant tumor of the cervix, although there was 1 other with early malignant change in the cervix.

After prolonged treatment with estrogens, the size of the testicle is reduced, the spermatic tubules are small and collapsed, and spermatogenesis is absent. Tumor formation in the testicle has not been observed.

The early effect of estrogenic stimulation of the prostate is an increase in the thickness of the lining epithelium in the prostatic urethra, which becomes increasingly keratinized. The fibromuscular stroma of the prostate is hypertrophied, and the glandular epithelium shows increase in the size of the cells and occasional dilation of acini. Later the fibromuscular stroma practically obliterates the glandular elements and the prostate shrinks in size. Cornification extends into the suburethral glands of the prostatic urethra, and there may be an irregular downgrowth of cells suggesting beginning transitional cell carcinoma in the prostatic urethra. Squamous cell cancer at this site or adenocarcinoma of the prostate has not been observed.

---

11. Mark, J., and Biskind, G. R.: Endocrinology **28**:465, 1941.

The thickening of the prostatic urethra leads to urinary obstruction. The ureters become dilated and thickened, and the bladder is also markedly distended and increased in size and thickness.

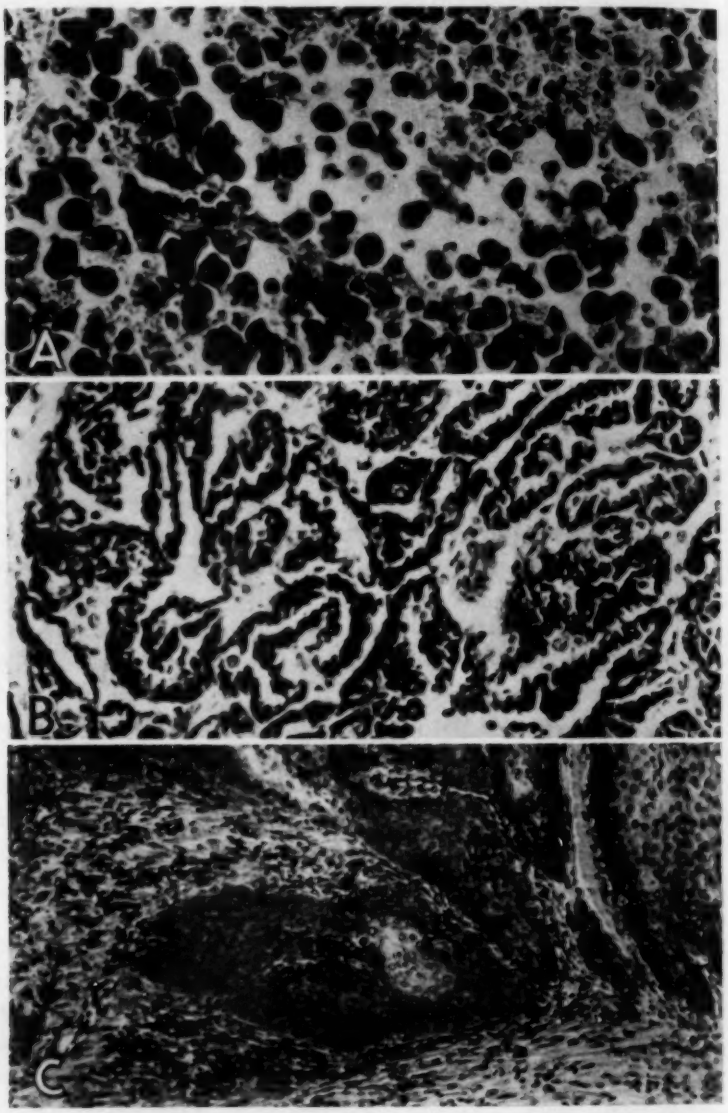


Fig. 5.—Cancers associated with the development of estrogenic mammary cancer in the rat: *A*, lymphosarcoma; *B*, cancer of the ovary; *C*, cancer of the cervix.

In the female genital tract keratinization and thickening occur in the mucous membrane of the vagina and cervix. These changes extend into the mucous glands and into the uterus, replacing the normal endo-

metrium. These changes are akin to those observed in the prostatic urethra. Carcinoma of the cervix arising at the base of the thickened cornified epithelium was found in 2 cases. Adenocarcinoma of the uterus was not observed.

Some workers (Cramer and Horning [7]) have sought to show that estrogenic cancer of the mammary gland is the result of changes in the endocrine system as a whole, particularly in the pituitary body and the adrenal glands, rather than a direct effect on the breast. These authors thought that they could inhibit mammary cancer by injecting a pituitary extract containing the thyrotropic factor. These results could not be confirmed by Asdell and Seidenstein [12] or by Lacassagne.[13] While we have been able to demonstrate that the presence of pituitary gland is necessary for the production of estrogenic mammary cancer, we have insufficient evidence to prove that the estrogenic effect on the mammary gland is an indirect one.

One of our experiments indicates that the growth of estrogenic mammary cancer may be partially inhibited by injections of anterior pituitary extracts. A group of 12 rats, 1 month old, received 10 micrograms of estradiol benzoate daily for a period of six months. At the end of one hundred days one third of the animals were autopsied and, on microscopic study of breast tissue, all of them were found to have mammary cancer. The remainder of the animals continued to receive their daily injections of the estrogen. In all but 2, which were reserved for controls, daily injections of anterior pituitary extract were added for the next four months. (These daily injections consisted of 5 rat units of a follicle-stimulating extract and 20 guinea pig units of a preparation containing the thyrotropic factor [ambinon, Roche-Organon] daily for one month followed by 10 international units of prolactin [Schering Corporation] daily for one month. Thereafter, these two cycles of injections of anterior pituitary extracts were each repeated for one month to complete the four months of treatment.) Palpable cancers developed in the animals which received only injections of estradiol benzoate. However, in the animals receiving in addition injections of anterior pituitary preparations palpable cancers were not found at autopsy, but all except 1 of the animals had microscopic cancers. Cramer,[14] who used injections of ambinon only, believed that the inhibiting effect of this anterior pituitary extract was more marked than indicated in this experiment.

---

12. Asdell, S. A., and Seidenstein, H. R.: Proc. Soc. Exper. Biol. & Med. **32**:931, 1935.

13. Lacassagne, A.: Am. J. Cancer **37**:414, 1939.

14. Cramer, W.: Am. J. Cancer **38**:463, 1940; Lancet **1**:192, 1939.

SUMMARY

All of the estrogens of sufficient potency for clinical use will produce mammary cancer in the rat regardless of chemical composition or physiologic potency. To produce mammary cancer, the dose must be well beyond the physiologic limit (ten or more times the threshold dose) and the treatment continuously applied for a period of months (thirty or more times the duration of normal estrus).

Variation in the time required to produce mammary cancer is dependent on: dosage, estrogenic potency, duration of estrogenic activity and method of administration. The total dose required to produce mammary cancer is not influenced by the amount of the daily dose but varies with the duration of estrogenic activity and the method of administration. It is difficult to demonstrate that sex, age or castration influences susceptibility to estrogenic mammary cancer. An initial period of estrogenic stimulation below the cancer-producing threshold renders the gland more susceptible to estrogenic cancer.

Important species differences are evident on the basis of experiments carried out in monkeys, rabbits and rats. A case of estrogenic mammary cancer in the rabbit is recorded.

Administration of testosterone or progesterone simultaneously with, or in sequence to, estrogenic stimulation does not prevent the appearance of mammary cancer. However, the growth of estrogenic cancer is inhibited by anterior pituitary extract.

To date, from a toxicologic standpoint the most important finding with respect to the estrogens is the production of mammary cancer by prolonged administration. In the clinical administration of the estrogens this toxicologic property of these compounds has not yet been verified but must be considered when compounds of high potency with prolonged activity are administered. The species difference remains an unknown factor.

Various changes in the endocrine glands accompany the appearance of mammary cancer, and cancerous change has been observed in other organs.

Preparations for use in this study were supplied by the manufacturers as follows: testosterone propionate, progesterone and estradiol dipropionate by the Ciba Pharmaceutical Products, Inc.; estradiol benzoate and a preparation of the anterior pituitary lactogenic factor (prolactin) by the Schering Corporation; an anterior pituitary extract containing the thyrotropic factor (ambinon) and estrone by Roche-Organon, Inc.; stilbestrol, stilbestrol dimethyl ether and stilbestrol monomethyl ether by Wallace & Tiernan Products, Inc.

Case Reports

MASSIVE FATTY INFILTRATION IN A COLLOID GOITER

R. G. Dhayagude, M.D., Bombay, India

Degenerative changes in a goiter are not uncommon. The usual types of degeneration met with are hyaline change, hemorrhage, calcification and sometimes ossification. Fatty change has been mentioned, but in the case to be reported here there appears to be an unusual example of a massive type of diffuse fatty infiltration in the stroma of a colloid goiter.

REPORT OF A CASE

A man aged 32 was admitted to the hospital because of a bilateral swelling of the neck of three and a half years' duration. Dr. V. M. Kaikini supplied the clinical notes for this report.

The history was that three and a half years before, a swelling appeared on the left side of the neck and gradually increased though at first the growth was slow and imperceptible. Six months prior to examination the mass started growing rapidly, became bilateral and assumed almost equal proportions on the two sides. Then slight difficulty of breathing developed. After that the patient felt weaker, lost weight and began to experience palpitations on exertion. He was fairly well built and nourished, although his nails and conjunctivas appeared to be pale. There was no history of any type of irradiation of this growth.

Local examination revealed a bilobular (horseshoe-shaped) bilateral swelling 7 by 4 by 4 inches (18 by 10 by 10 cm.) in dimension, situated below the thyroid cartilage, astride the trachea and cricoid. It moved with deglutition. It was soft in consistency but did not show any fluctuation. The overlying skin was free, and no prominent veins could be seen over it. Transmitted pulsation of the carotid arteries could be felt over the tumor, but no systolic bruit could be heard. Lymphatic glands were not palpable. He had no stridor, but his voice was slightly hoarse. He presented a little bilateral proptosis, but none of the eyelid signs, such as Möbius' or Stellwag's, could be elicited. There were tremors. The palpebral fissure was slightly wider than usual. Direct laryngoscopic examination showed abductor paresis of the right vocal cord. The pulse rate was 80 per minute, the temperature 97 F. and the respiratory rate 22 per minute. No other abnormality could be detected on physical examination. A diagnosis of colloid goiter undergoing a malignant change was made, and the whole of the right half of the thyroid was removed. As the case appeared to be one of carcinoma, the wound was closed in layers and sutures were placed. In the evening the patient developed coarse rales on both sides of the chest; his pulse became fast (168 beats per minute) and feeble. The temperature began to rise and the next day the pulse became poor in volume, and the man died with a temperature of 104 F. Unfortunately, permission for an autopsy was not obtained.

Examination with the Naked Eye.—The weight of the thyroid tissue removed was 500 Gm. It was received in the department cut into two irregularly rounded

From the Department of Pathology and Bacteriology, Seth Gordhandas Sunderdas Medical College.

masses constituting the right lobe and a part of the isthmus of the gland. One of them was bigger, and its dimensions were 10 by 8 by 5.5 cm.; the smaller one measured 8 by 6 by 5.5 cm. The smaller represented in major part the isthmus of the gland, which showed one or two nodules on the surface. The rest of it had a smooth surface, which, however, showed a few dilated veins. The bigger piece was more nodular and had large areas of brownish accumulations of colloid shining through the capsule. It also presented a number of dilated veins on the surface. When cut into, it showed numerous irregular areas of accumulations of transparent ambered-colored colloid material (fig. 1). Some of the areas were as small as a pea, while others were larger than 2 by 2.5 cm. on cross section. The colloid was generally homogeneous, but in some of the vesicles it had taken an opaque yellowish brown hue, probably due to hemorrhage. The areas containing colloid varied in size and shape and were separated in places by thin septums while in other places groups of these areas were separated by strands of yellowish white soft tissue. During the process of cutting, or as a result of little pressure, the masses of colloid had dropped out of the vesicles, leaving smooth-lined cavities. The tissue from the isthmian region showed few colloid areas and a large amount of yellowish white solid soft tissue.

Histologic Examination.—Paraffin sections stained with hematoxylin and eosin showed a very unusual structure. Sections from the solid-looking areas near the isthmus showed an appearance which superficially could not be recognized as thyroid structure (fig. 2). It consisted mostly of fatty areolar tissue, in which on careful scrutiny a few isolated vesicles containing colloid could be identified. The thyroid acini were irregular in size and shape and generally contained abundant colloid, which filled the whole of the acinus except at the periphery, where it showed a vacuolated structure or a clear area due to shrinkage of the colloid. The colloid took a uniform hyaline pinkish color and microscopically appeared normal. In many of the acini a number of desquamated epithelial cells were found in the colloid material. The epithelial cells lining the acini generally formed a single row of cuboid cells. The cytoplasm and nuclei of the cells showed normal structure.

Sections from the colloid-bearing areas of the right lobe of the thyroid gland showed enormously dilated irregular acini holding masses of colloid material, which in many of the acini had shrunken from the wall. Some of the acini showed evidence of hemorrhage into their lumens and also outside into the fatty areolar stroma. The latter in these areas showed deposition of large numbers of hematoidin and hemosiderin granules both within and without the cells. The septums dividing the enormously dilated, giant acini were thin and sometimes consisted of nothing but a strip of connective tissue lined on each side by epithelial cells, which did not evince any marked proliferative activity (fig. 3). The blood vessels did not show any abnormal change.

Sections stained by Masson's [1] trichrome stain for connective tissue showed an enormous increase in fibrous connective tissue, forming a vacuolated reticular structure. The vacuoles had contained fat, which dissolved out during the preparation of the sections.

In order to be assured of the presence of fat and if possible to determine the chemical nature of the fat, frozen sections were cut and stained by sudan III and scarlet red. These preparations (fig. 4) showed that the vacuolated areas in between the fibrous connective tissue strands contained a large amount of neutral fat globules which had taken a yellow or a red color, according to the stain used.

---

1. Masson, P.: J. Tech. Methods **12**:75, 1929.

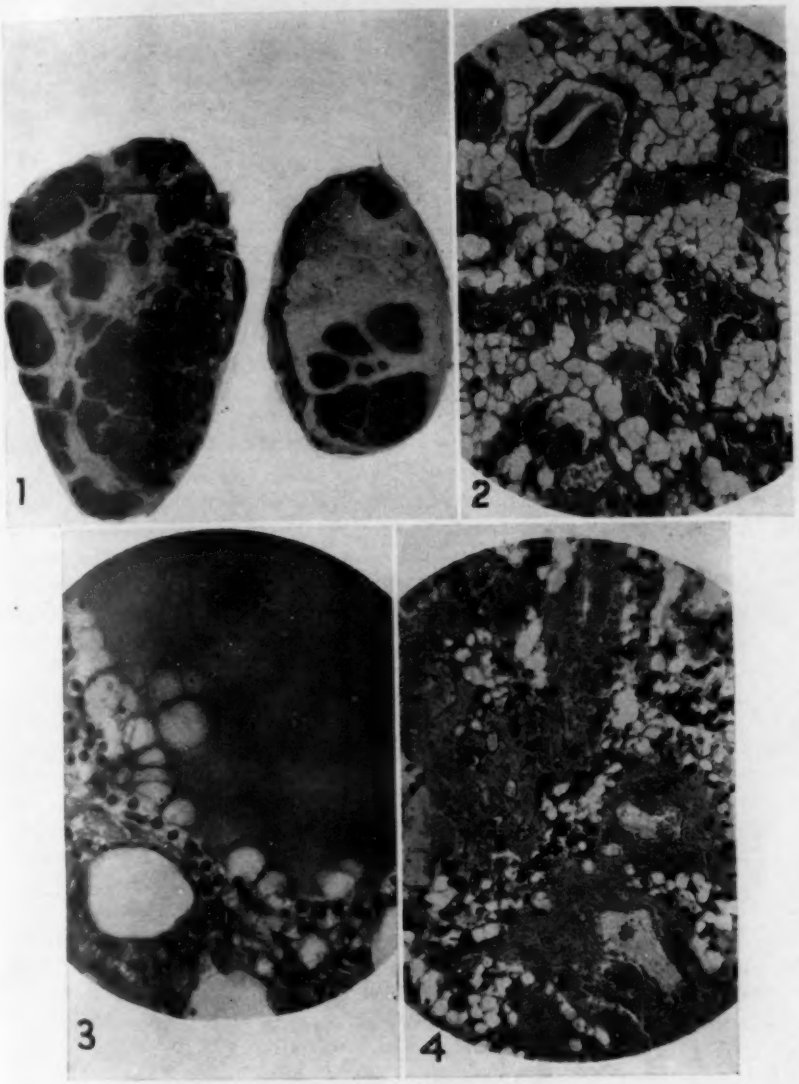


Fig. 1.—Photograph of transverse sections of the specimen showing large black areas of amber-colored colloid material, well seen in the larger of the two sections. The whitish areas in the small section represent fibrofatty infiltration.

Fig. 2.—A low power photomicrograph of a section showing large areas of fibrofatty infiltration and a single thyroid vesicle containing colloid material.

Fig. 3.—A high power photomicrograph depiciting a large thyroid vesicle lined by a single layer of cuboidal epithelium.

Fig. 4.—A frozen section stained with sudan III. The black dots represent fat globules.

SUMMARY

A case is reported of an extensive fatty infiltration in the stroma of a colloid goiter. The rapidity of growth, probably due to the infiltration, led to a clinical suspicion of malignancy. The fatty change was so massive that it produced pressure symptoms causing abductor paralysis of the right vocal cord.

OBSTRUCTING SECONDARY CARCINOMA OF THE ESOPHAGUS

PAUL GROSS, M.D., AND LESTER J. FREEDMAN, M.D., PITTSBURGH

Metastatic tumor involving the esophagus is exceedingly rare. Willis [1] found only three reports of cases in the literature. These were the report by von Recklinghausen [2] in 1885 of a tumor of the tibia diagnosed as myxochondrosarcoma, with a pea-sized nodule in the muscularis of the esophagus, Spiegelberg's [3] report of multiple nodules in the esophageal mucosa metastatic from melanosarcoma of the eye and Prym's [4] report of an esophageal metastasis from testicular chorioncarcinoma. In the latter case the metastasis involved the muscularis and caused elevation of the mucosa. It was the size of a cherry seed and was situated above the tracheal bifurcation.

Steiner [5] reported 2 cases in which carcinoma of the esophagus was associated with carcinoma of the vallecula. In each instance he assumed, without adequate evidence, that the tumor of the esophagus was primary and that of the vallecula secondary. Guisez [6] cited 5 cases. Although no details were given, the involvement in 2 was probably by direct extension, and there is a possibility that direct extension played a role in the other 3.

The largest contribution to this subject was made by Zuppinger [7] and Proske [8] who reported on the same material in separate publications. Their clinical material included 382 cases of mesopharyngeal and hypopharyngeal carcinoma, among which were 21 cases with associated esophageal carcinoma. In this study 2,500 cases of tumor (malignant?) encountered over a sixteen year period were investigated, but concomitant esophageal carcinoma was found only in cases of pharyngeal or laryngeal carcinoma. Although detailed information on these cases was not given, it was assumed in every instance that the esophageal tumor was secondary. The evidence in support of this assumption was not conclusive. In 10 of the 21 cases biopsy was done, and in 9 the histologic appearance of the two tumors was similar. In 1 case the "primary" tumor was said to have been keratinizing epidermoid carcinoma, while the "secondary" tumor was "anepidermoides carcinoma." Zup-

From the Western Pennsylvania Hospital Institute of Pathology and the Department of Radiation and Physical Therapy.

1. Willis, R. A.: The Spread of Tumours in the Human Body, London, J. & A. Churchill, 1934.
2. von Recklinghausen, F.: Virchows Arch. f. path. Anat. **100**:503, 1885.
3. Spiegelberg, H.: Virchows Arch. f. path. Anat. **142**:553, 1895.
4. Prym, P.: Beitr. z. path. Anat. u. z. allg. Path. **85**:703, 1930.
5. Steiner, O.: Med. Klin. **18**:1249, 1922.
6. Guisez, J.: J. Laryng. & Otol. **40**:213, 1925.
7. Zuppinger, A.: Ztschr. f. Krebsforsch. **47**:413, 1938.
8. Proske, E.: Strahlentherapie **64**:227, 1939.

pinger [7] concluded that every seventh or eighth patient with carcinoma of the mesopharynx or hypopharynx cured by radiation therapy will die from secondary esophageal carcinoma. A second, equally startling conclusion was that in practically every instance the esophageal tumor was initiated by implantation metastasis. The lymphogenous route was considered rare.

A tabulation prepared in the tumor clinic of the Stanford University School of Medicine [9] lists 11 esophageal metastases in 1000 cases of carcinoma in which autopsy was done. In 6 cases the neoplasm was primary in the stomach; in 2 in the larynx, and in 3, in the breast, the bronchus and tongue, respectively. No case had been described in which obstruction resulted from metastatic carcinoma of the esophagus. The present case of secondary carcinoma of the esophagus with obstruction is deemed worthy of record because of the rarity of this finding.

REPORT OF A CASE

A 71 year old white man was admitted to the Western Pennsylvania Hospital with the complaints of vomiting, loss of weight (70 pounds [31.8 Kg.]) and anorexia of one year's duration. The onset had been gradual, with occasional regurgitation several minutes after swallowing. At the time of admission vomiting was frequent, occurring immediately, as well as several hours, after meals. The vomitus had no sour taste and frequently contained undigested food eaten one or more days previously. There was no associated nausea or pain, although a sensation of obstruction behind the lower part of the sternum was present after swallowing. Of significance in the past history was the fact that during the past ten years extreme frequency of urination had developed, with occasional burning.

The patient appeared listless but in no distress. The skin was inelastic and hung in loose folds. The thorax was barrel shaped. No abnormality was found in the chest or in the abdomen. The prostate was smooth, firm, moderately enlarged and not tender. The temperature and the pulse and respiratory rates were normal. Laboratory tests furnished no significant information.

Roentgenologic examination of the esophagus showed complete obstruction in the middle third. The barium sulfate silhouette at the constriction was smooth, conical and central. Slight dilatation was present proximal to the obstruction. Peristaltic waves coursed down the esophagus but stopped at the constriction. The hilar nodes were enlarged; they filled the aortic window and surrounded the affected esophageal area. There was partial atelectasis in the lower lobe of the right lung near the cardiophrenic angle. Consideration was given to the possibility that the esophageal obstruction was secondary to extrinsic pressure of enlarged hilar nodes.

After a therapeutic test with roentgen rays, the esophageal obstruction did not improve, nor did the lymph nodes diminish in size. A tentative impression of lymphoblastoma was therefore discarded. Gastrostomy was performed, and roentgen therapy was continued until a full course [10] was administered. This was

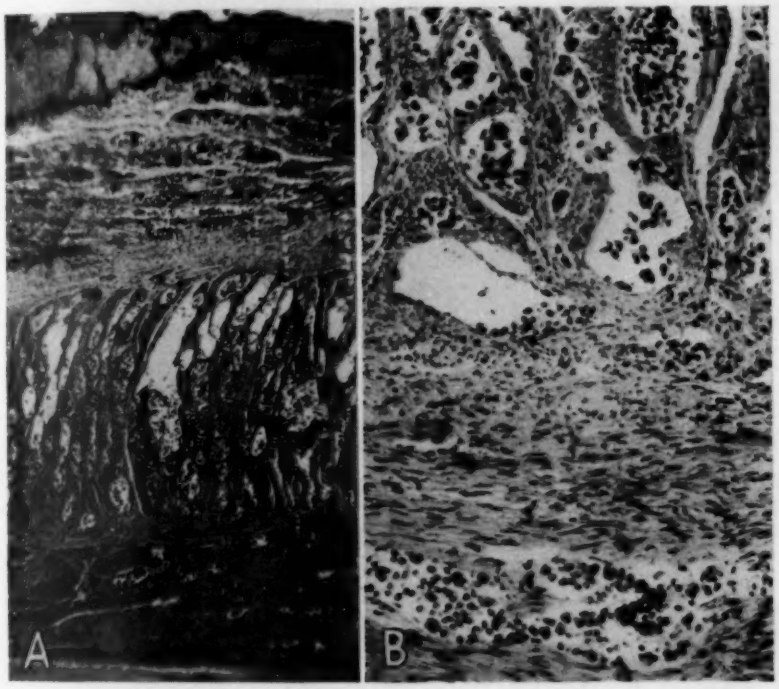
9. Diagnosis and Treatment of Malignant Tumors, Syllabus for Postgraduate Course, San Francisco, Tumor Clinic, Stanford University School of Medicine, 1937.

10. The treatment formula was as follows: 200 r, measured in air, given daily except Sundays to both an anterior and a posterior mediastinal portal, with 200 kilovolts, 0.5 mm. copper and 3 mm. aluminum filtration and a 50 cm. target-skin distance, at the rate of 16 r per minute. The therapeutic test dose was 800 r per portal. The total dose (skin tolerance) was 1,800 r per portal.

done on the basis of the clinical impression that the esophageal carcinoma was primary and had metastasized to the hilar lymph nodes. Following this treatment, an unusual picture was seen roentgenologically. Instead of the expected ragged eccentric shadow, a perfectly smooth central one was observed as the barium sulfate passed through the esophageal constriction.

Death occurred ten weeks after admission.

The autopsy disclosed a firm mass at the junction of the lower and middle thirds of the esophagus. It encircled the wall but did not increase its external diameter. The mass was 3 cm. long and almost, but not quite, obliterated the lumen. The mucosa was intact and normal over this mass. A pronounced increase



A, photomicrograph of a longitudinal section of the esophagus through the region of constriction. There is a pronounced increase in the thickness of the submucosa and the muscularis caused by tumor cells. In the inner circular muscle coat there are large and small cystic spaces which contain scattered tumor cells. Hematoxylin and eosin; × 16. *B*, higher magnification of the muscularis. Hematoxylin and eosin; × 95.

in the thickness of the esophageal wall (7-8 mm.) was caused predominantly by the muscular coats, the cut surface of which bulged and presented a smooth, glistening and almost translucent appearance. This tissue was firm, almost hard. The other coats retracted and did not appear greatly increased in thickness. Above and below this mass the esophageal wall appeared normal.

The prostate was symmetrically enlarged. The medial lobe projected 2 cm. into the bladder. The consistency was firm, but on section no striking abnormality was noted.

Small, white, firm, raised nodules, 1 to 4 mm. in diameter, were noted on the visceral pleuras and in the lungs, particularly in the hilar regions. Similar nodules, some of them up to 1 cm. in diameter, were seen on the anterior aspect of the stomach. These nodules were limited to the peritoneum and did not involve the other coats. The gastric mucosa was normal, and the gastrostomy opening was patent. The tracheobronchial nodes were enlarged, up to 2 cm. in diameter, deeply anthracotic and very firm in consistency.

Microscopically, sections of the esophagus through the constricting mass showed a pronounced increase in the thickness of the submucosa and the muscular coats because of the presence of tumor cell masses, tumor-containing cystic spaces and edema (*A* in figure). The cystic spaces were of irregular or oval shape, often large, and contained loosely scattered epithelial cells or oval glands. The tumor cells varied considerably in size and shape. On the whole, they were small, with relatively large, irregular and often hyperchromatic nuclei and a relatively small amount of pale cytoplasm. Less numerous and smaller tumor cell masses and nests were present in the adventitia, which showed an increased amount of fibrous tissue. The mucosa was intact but showed focal epithelial hyperplasia.

Sections of the prostate gland showed several isolated and relatively small foci of adenocarcinoma. Two types of tumor tissue were seen. In one the glands were composed of darkly staining cells, similar to those found in the esophagus, and in the other the glands were composed of relatively clear cells with smaller, fairly uniform nuclei.

The tumor metastases in the trachea, bronchi, lung parenchyma, lymph nodes and peritoneum were similar to the tumor found in the esophagus.

COMMENT

This case is the first recorded instance of an obstructing secondary tumor of the esophagus. There is added interest in the fact that the neoplasm simulated a primary tumor and was diagnosed as such roentgenologically. Because of debility, esophagoscopy could not be performed. No doubt, had it been feasible, esophagoscopy would have ruled out the possibility of a primary neoplasm on the basis of an intact normal mucosa in the area of constriction.

SUMMARY

Obstructing secondary carcinoma of the esophagus, metastatic from clinically silent prostatic carcinoma, in a 71 year old man is reported. This tumor simulated a primary tumor clinically. The significant roentgenologic finding was a smooth central filling defect in the barium sulfate column.

CONGENITAL IDIOPATHIC CARDIAC HYPERTROPHY

SYDNEY J. WEISMAN, M.D., MINNEAPOLIS

The infrequent occurrence and the obscurity of the causes of congenital idiopathic cardiac hypertrophy have led to reports of single cases and to many discussions of the etiologic, pathogenic and other aspects of the anomaly. In 1933 Kugel and Stoloff [1] reviewed the literature and found that 52 instances had been reported up to 1932. They classified all the cases into three groups. Group I included cases of pure hypertrophy of the myocardium with no other pathologic change and with no recognizable cause; group II, cases of cardiac hypertrophy with other changes in the myocardium, and group III, cases in which complete examination was not reported and which therefore were questionable. Kugel and Stoloff included 17 cases in group I, 27 in group II and 8 in group III. They stated that "when the knowledge of heart disease in children increases so that it equals the present knowledge of heart disease in adults, the term congenital idiopathic hypertrophy of the heart may disappear from the literature." They reported 7 more cases of their own, in 1 of which they observed, in addition, renal disease and anemia. In all 7 there was replacement fibrosis in the myocardium, and in most there was endocardial and perivascular fibrosis. They accepted as authentic, however, many cases in which endocardial fibrosis was observed.

Apparently the first authentic instance was reported by Simmonds [2] in 1899. The condition was noted in a newborn infant who died immediately after a prolonged delivery. Since the review by Kugel and Stoloff, many case reports have appeared in the literature. Kenny and Sanes [3] added 2 cases but thought that in 1 the condition was due to parenchymatous myocarditis. The patient whose case was reported by MacMahon [4] died in uremia due to congenital folds in the ureters. In Levine's [5] report there was mention of definite coarctation of the aorta. The cases of Benjamin and Simon,[6] Amberg and Willius,[7] Dammin and Moore,[8] Kugel [9] and Powers and LeCompte [10] seemed

From the Department of Pathology of the University of Minnesota Medical School.

1. Kugel, M. A., and Stoloff, E. G.: Am. J. Dis. Child. **45**:828, 1933.
2. Simmonds, M.: München. med. Wchnschr. **46**:108, 1899.
3. Kenny, F. E., and Sanes, S.: J. Pediat. **3**:321, 1933.
4. MacMahon, H. E.: Am. J. Path. **13**:845, 1937.
5. Levine, H. D.: Am. J. Dis. Child. **48**:1072, 1934.
6. Benjamin, B., and Simon, M. A.: Am. J. Dis. Child. **59**:842, 1940.
7. Amberg, S., and Willius, F. A.: Proc. Staff Meet., Mayo Clin. **13**:470, 1938.
8. Dammin, G. J., and Moore, R. A.: Arch. Path. **27**:122, 1939.
9. Kugel, M. A.: Am. Heart J. **17**:602, 1939.
10. Powers, G. F., and LeCompte, P. M.: J. Pediat. **13**:760, 1938.

to be authentic; however, in many there were some other changes in the myocardium. Lightwood and Court's [11] case was diagnosed only by clinical study and the patient is said now to be improving. The 2 cases reported by Mahon [12] are apparently cases of true idiopathic hypertrophy. In Ellis' [13] case the condition was probably due to glycogen storage. Bland, White and Garland [14] assumed that the cardiac hypertrophy in their case was due to an anomaly of the coronary circulation, but later it was found to be due to a glycogen storage disease. In 1936 Mahon [12] reported 16 more cases recorded in the literature since the review by Kugel and Stoloff. Of the entire total of cases in which autopsies were made up to 1936 (62), he found 22 in which there was also shown endocardial fibrosis and myocardial degenerative and inflammatory changes. Other cases of congenital idiopathic hypertrophy of the heart have been reported recently, but many of the reports are in foreign journals not available.

All the patients whose cases have been described were children in the early years of life, and most were within the first year. There has been no remarkable difference in the sex distribution. The accompany-

Age Distribution of Seventy Cases of Congenital Cardiac Hypertrophy

Age	Cases	Percentage
Up to 1 mo.	4	6
1 - 5 mo.	19	27
6 - 11 mo.	26	37
12 - 23 mo.	12	17
2 - 5 yr.	6	8.6
6 - 9 yr.	3	4.3

ing table shows the age distribution of 70 cases reported in the literature. Seventy per cent of the patients were infants under 1 year of age.

In most of the cases death has come suddenly or has occurred shortly after periods of dyspnea and cyanosis in previously apparently normal infants.

Sanes and Kenny [15] reported a case of congenital cardiac hypertrophy with the left coronary artery arising from the pulmonary artery. The role of an anomalous left coronary artery in congenital idopathic hypertrophy has precipitated much argument concerning the cause of the hypertrophy. Abrikosoff,[16] Heitzmann [17] and Scholte [18] each have reported such anomalies, but only the left ventricle was affected and

11. Lightwood, R., and Court, D.: Proc. Roy. Soc. Med. **32**:316, 1939.
12. Mahon, G. S.: Am. Heart J. **12**:608, 1936.
13. Ellis, R. W. B.: Proc. Roy. Soc. Med. **28**:1330, 1935.
14. Bland, E. F.; White, P. D., and Garland, J.: Am. Heart J. **8**:787, 1933.
15. Sanes, S., and Kenny, F. E.: Am. J. Dis. Child. **48**:113, 1934.
16. Abrikosoff, A.: Virchows Arch. f. path. Anat. **203**:413, 1911.
17. Heitzmann, O.: Virchows Arch. f. path. Anat. **223**:57, 1917.
18. Scholte, A. J.: Centralbl. f. allg. Path. u. path. Anat. **50**:183, 1930.

showed aneurysmal dilation in some part along with fibrotic changes in the myocardium. Similar anomalies of the left coronary artery have been reported by Kiyokawa [19] and by Carrington and Krumbhaar.[20] In these cases, also, the pathologic change was primarily in the left ventricle, and although hypertrophy and dilation were present, there was also considerable myocardial degeneration as well as fibrosis.

According to the opinion expressed by Kugel and Stoloff, the heart with true idiopathic hypertrophy shows no myocardial degeneration or fibrosis. Dammin and Moore [8] found an apparent increase in the number of fibers and no increase in the number of nuclei. In normal growth they found an increase in the total number of nuclei and of fibers. These investigators concluded that hypertrophy in the infant's heart is due to an increase in the length and the width of the fibers, whereas hypertrophy in the adult's heart is due to an increase in the width alone; in neither is there an increase in the number of nuclei. MacMahon,[21] however, pointed out that in cardiac hypertrophy appearing at an early age mitotic figures may be seen which prove the true proliferation of the muscular elements.

Steiner and Bogin [22] found no relation between so-called status thymicolymphaticus and congenital idiopathic cardiac hypertrophy.

REPORT OF CASES

Case 1.—The patient was a white girl 7 weeks of age. The birth and subsequent history were normal. There had been no cyanosis or cough, and the gain in weight had been normal. The infant attended a "well baby" clinic. The last examination had been made the day before death, and the infant was considered healthy. On April 12, 1941 the mother noted that the baby did not feel well at noon. At 3 p. m. the temperature was subnormal, and the infant was irritable. At 6 p. m. the baby began to have difficulty in breathing, grunting with each inspiration. The physician arrived at 7:30 p. m. By this time the infant was markedly cyanotic, and the respirations were labored. The temperature was 103.8 F.; the pulse rate, 160 and regular; the respirations, 50 to 60 per minute. The patient was hospitalized immediately and placed in an oxygen tent. The cyanosis disappeared to some extent. Shortly thereafter the respirations began to fail again, and the cyanosis reappeared. Alpha lobelin was administered, with a good response; the respirations became more normal, and the cyanosis disappeared to some extent. A roentgenogram of the chest made at this time showed marked enlargement of the cardiac shadow (fig. 1*A*). Again the respirations became shallow, although the respiratory passages were demonstrated to be patent. The infant died at 11 p. m. Throat cultures taken after death were negative for diphtheria bacilli.

The autopsy was made nine and one-half hours after death. The body was that of a well developed and well nourished white girl. The weight was about 4,500 Gm., the crown-heel length 56 cm., the crown-rump length 41 cm., the circum-

19. Kiyokawa, W.: Virchows Arch. f. path. Anat. **242**:14, 1923.

20. Carrington, G. L., and Krumbhaar, E. B.: Am. J. Dis. Child. **27**:449, 1924.

21. MacMahon, H. E.: Am. J. Dis. Child. **55**:93, 1938.

22. Steiner, M., and Bogin, M.: Am. J. Dis. Child. **39**:1255, 1930.

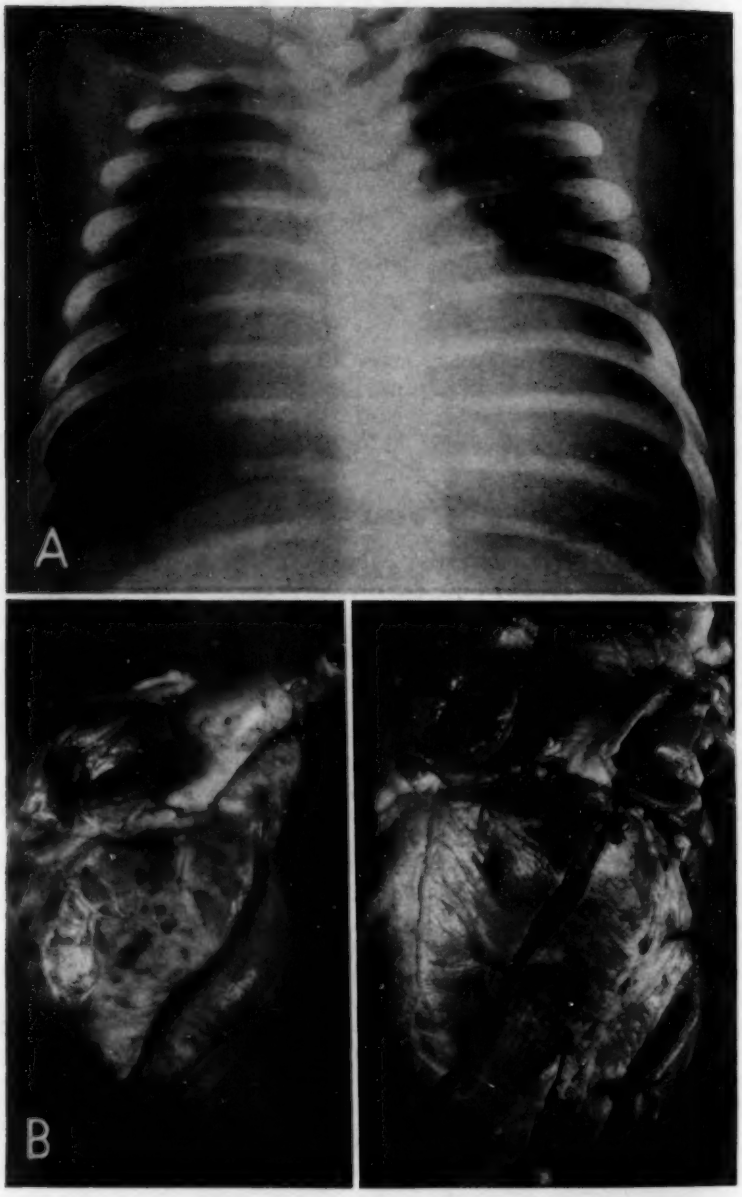


Fig. 1 (case 1).—*A*, roentgenogram taken a few hours before death. *B*, heart (right) of the patient in comparison with a normal heart (left) from an infant of the same age.

ference of the head 39 cm. and the circumference of the chest 35 cm. There was cyanosis of the lips and finger nails. The peritoneal and pericardial cavities contained a slight excess of clear yellow fluid. The edge of the liver was 3 cm. below the right costal margin.

The heart measured 5 cm. in its greatest transverse diameter and 6 cm. from the base of the pulmonary artery to the apex. The heart weighed 50 Gm. (fig. 1*B* [right]). There were numerous subepicardial petechiae: two along the junction of the left auricle and left ventricle, six along the posterior surface of the interventricular septum and one at the tip of the right auricle. There was considerable dilation of the left auricle and left ventricle as well as moderate hypertrophy of the left ventricle. There was moderate dilation of the right auricle and right ventricle with some hypertrophy of the right ventricle. The myocardium

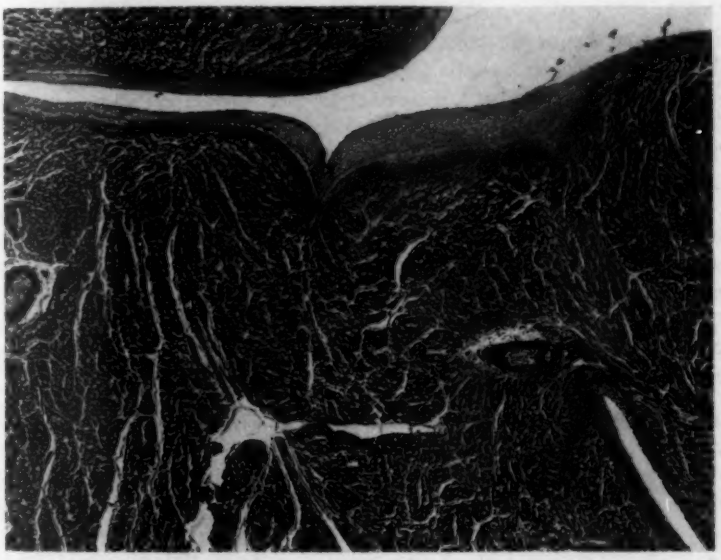


Fig. 2 (case 1).—Photomicrograph of myocardium and endocardium; × 35.

of the left ventricle measured 7 mm. in its greatest thickness and that of the right ventricle 5 mm. The endocardium was grayish white and in most portions showed great thickening. At the level of the upper ends of the papillary muscles of the left ventricle the endocardium was 5 mm. thick. The papillary muscles were well covered with thick endocardium, and the cut surfaces of the muscles showed in many areas grayish white streaks through the whole transverse diameter with no evidence of the normal redness of the myocardium in them. This same process was present in scattered areas throughout the myocardium, and the streaks were always continuous with the endocardium. The thickening of the endocardium was most marked over the interventricular septum, in the upper portion of the right ventricle and in the apical portions of the right and left ventricles. There were no abnormalities of the valves, and the valvular endocardium was of normal thickness. Both coronary arteries arose from the aorta, were patent throughout and showed no abnormalities in their courses. The ductus arteriosus was almost

completely obliterated. The foramen ovale was closed, and there were no abnormal communications between the chambers. The trabeculae carneae appeared flattened owing to the great thickness of the endocardium.

The bronchi in the lower lobe of the left lung and in the middle and lower lobes of the right lung contained frothy mucoid fluid. There was moderate hypostatic congestion inferiorly and posteriorly.

The liver weighed 200 Gm. The capsular surface appeared normal. The cut surfaces showed the "nutmeg" appearance of chronic passive congestion.

There were three small unilocular cysts in the right ovary containing clear yellow fluid.

The other organs showed no noteworthy changes.

Microscopically the liver showed moderate chronic passive congestion with scattered areas of atrophy of the hepatic cords about the central veins. No changes occurred in the portal spaces.

In the lower part of the lungs were scattered areas of atelectasis. Many alveoli contained large mononuclear cells which had not ingested hemosiderin. These cells appeared to be septal cells that had rounded off and become free; all transitions were traced. No exudate was found in the alveoli.

There was severe thickening of the endocardium with fibrous and elastic tissue (fig. 2). There was wide separation of the muscle fibers, with empty spaces between them. There was no apparent hypertrophy of the fibers. No cellular infiltrate, no degenerative changes, no perivascular or myocardial fibrosis appeared. The myocardium was negative for fibrosis and for glycogen with special stains. The stain for glycogen was applied to tissue fixed in absolute alcohol. The grayish white streaks in the myocardium apparently represented the empty spaces seen on microscopic examination.

Sections of the other organs showed no noteworthy changes.

Case 2 (reported with the permission of Dr. A. H. Wells of Duluth, Minn.).—The patient was normal for the first two weeks of life. During the next two weeks there were periods of cyanosis which usually were of short duration. This infant was found dead in bed. An enlargement of the heart had been recognized before death.

The heart weighed 40 Gm. There were hypertrophy and dilation of the right and left ventricles, hypertrophy and dilation of the left auricle and dilation of the right auricle. The myocardium of the left ventricle measured 7 mm. in its thickest portion and that of the right ventricle 5 mm. The endocardium was thickened in all chambers but most markedly in the left ventricle. The trabeculae carneae appeared flattened. There were no abnormalities of the coronary arteries. The lungs showed chronic passive congestion. The remainder of the examination gave negative results.

Microscopic sections of the lung showed septal cells swollen, often free in the alveoli, and the septal capillaries engorged with blood. There was no exudate in the alveoli.

There was moderate thickening of the endocardium with a small amount of subendocardial fibrosis. Muscle fibers were separated, with empty spaces between them, but at times there was definite myocardial fibrosis. There was perivascular fibrosis. A few of the fibers showed a loss of sarcoplasm about the nuclei with a vacuolated appearance. There was no generalized degeneration of fibers. Scattered

areas of myocardial atrophy were present, however. No hypertrophy of the fibers was apparent. There was no cellular infiltrate. Sections stained for glycogen were not available.

The other organs revealed nothing of note.

SUMMARY

Congenital idiopathic cardiac hypertrophy is enlargement of the heart by hypertrophy and dilation, associated frequently with thickening of the endocardium. The true cases, according to Kugel and Stoloff, show no myocardial changes, although perivascular fibrosis has been described frequently by others. No other lesion or anomaly is present in the true cases. Too few cases of anomaly of the coronary circulation have been described along with idiopathic hypertrophy to support any causal relation. When such an anomaly is present, the resulting changes have not been the same as in true idiopathic hypertrophy. It seems logical to assume that the association of the two defects is accidental. Fetal myocarditis has not yet been proved to be the etiologic factor in the hypertrophy. Although, as knowledge of abnormal conditions of the infant heart increases, fewer cases of cardiac hypertrophy are considered cases of idiopathic hypertrophy, there still remain many which cannot be explained.

CHRONIC OCCLUSION OF PORTAL VEIN

Report of Two Cases, One a Case of Occlusion Associated with Aneurysm of the Splenic Artery and Carcinoma of the Liver (Hepatoma)

MILTON D. BOSSE, M.D., AND JAMES M. STRANG, M.D., PITTSBURGH

Chronic occlusion of the portal vein is uncommon. The most comprehensive review on this subject was published by Simonds [1] in 1936. In addition to the 95 cases reviewed by Simonds, including 1 case of his own, 23 others have been found described in the literature. Because of the rarity of chronic occlusion of the portal vein, 2 cases are now reported.

CASE 1.—A white man 58 years old was admitted, complaining of weakness and a loss of 30 pounds (13.5 Kg.) in weight during the previous three months. His blood pressure was 150 systolic and 110 diastolic. The liver was nodular. The Kahn test of the blood was negative; the icterus index, 5; the stool, strongly positive for occult blood; a Wallace-Diamond test made on the urine was positive in a dilution of 1:80.

Roentgenologic examination after a barium sulfate meal revealed an irregular calcific shadow, 6 cm. long and 3 cm. wide, just to the left of the first and second lumbar vertebrae. It was outside the stomach, spleen and kidney. Another irregular calcific shadow, 13.5 cm. long and 1 to 2.5 cm. wide, was seen to the right of the first, second and third lumbar vertebrae.

No other significant findings were obtained from the history, the record of the physical examination or the reports of many other laboratory tests. The patient's condition gradually became worse, and he died twenty-four days after admission.

Autopsy (three and one-half hours after death).—The body was that of an emaciated elderly white man. No free fluid was found in the peritoneal or the pleural cavity.

The liver was irregular in shape, yellowish brown, nodular, and weighed 1,400 Gm. It exhibited very extensive infiltration, especially of the right lobe, by tumor nodules, which varied from 7 cm. to a few millimeters in size. The color of the tumor varied from homogeneous gray to yellowish white or was very hemorrhagic, and the consistency of the tumor varied from firm to soft. The gallbladder and the biliary passages were normal. No evidence of tumor was found elsewhere in the body.

The veins in the wall of the midportion of the esophagus were visible through the mucosa and were moderately dilated. The appendix was 7 cm. long and 0.5 cm. in diameter. It was bound down to the posterior wall of the cecum by thin fibrous tissue. A mucocele, 0.5 cm. in diameter, was present at the tip, but elsewhere the lumen of the appendix was nearly obliterated.

From the Institute of Pathology (Ralph R. Mellon, M.D., director) and the Medical Division of the Western Pennsylvania Hospital.

1. Simonds, J. P.: Arch. Surg. **33**:397, 1936.

The portal and the superior mesenteric vein together formed a continuous, elongated, apparently calcified or ossified mass from 1 to 2.5 cm. thick (fig. 1). On section the lumens were slitlike, eccentrically placed and surrounded by calcific material. One of the mesenteric venous tributaries from the appendical region which joined the calcific portal vein was calcified throughout its course. The other mesenteric tributaries appeared normal. The splenic vein was moderately dilated but showed no calcification. The vein from the lesser curvature of the stomach

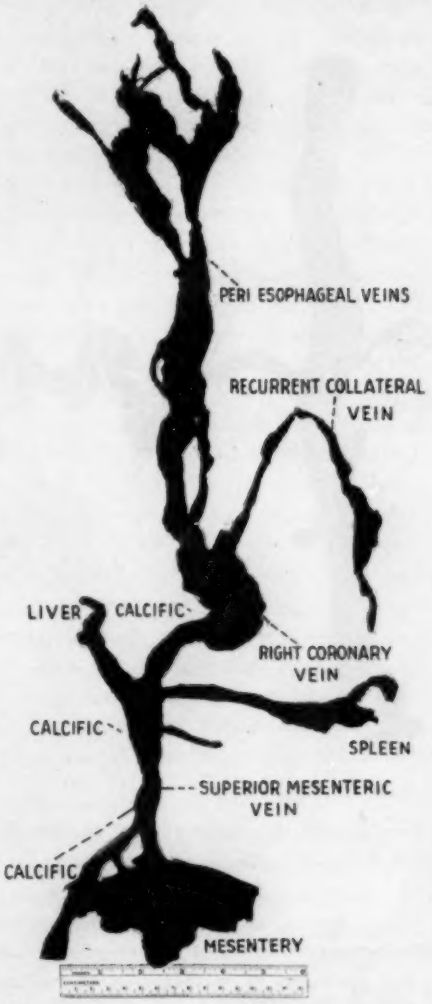


Fig. 1.—Portal venous system in case 1. Note the large calcific saccular dilatation of the right coronary vein and the very large periesophageal veins.

(the coronary vein of the stomach) was greatly dilated, and about 6 cm. above the junction with the main portal stem it formed an "egg shell-like" structure, 6 by 4 by 3 cm. (fig. 1). Dilated periesophageal and other enlarged collateral veins originated from the upper pole of this structure.

The aorta and its branches showed moderate atherosclerosis. The splenic artery was tortuous, coiled on itself, and presented a uniformly cylindric aneurysmal dilatation throughout most of its length. It was 1.5 to 2 cm. in diameter and 55.5 cm. long when straightened (fig. 2). The wall was sclerotic and antemortem thrombi partially occluded the lumen. About 6 cm. before entering the spleen, the artery divided into a number of coiled branches.

Microscopic sections of the tumor in the liver showed considerable variation in size, shape and staining of the cells. Numerous hyperchromatic and large nuclei,

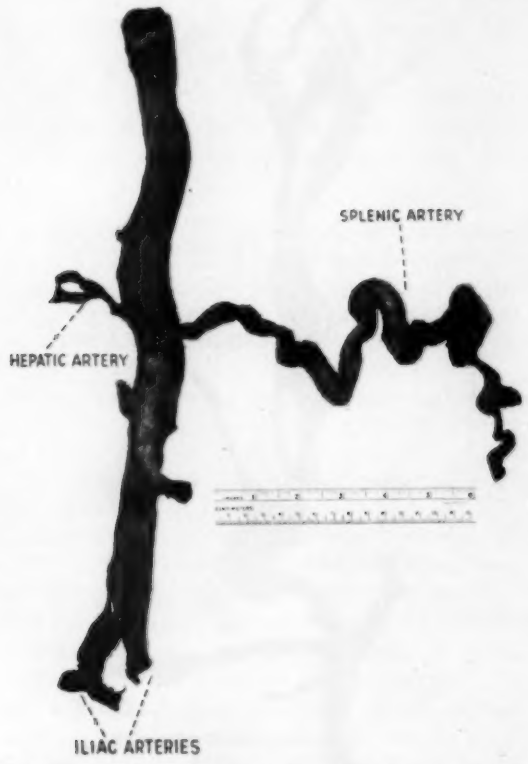


Fig. 2.—Cirsoid aneurysm of the splenic artery in case 1. The vessel when straightened was longer than the aorta.

as well as scattered mitotic figures, were seen. In some portions there were large cells with eosinophilic cytoplasm arranged in irregular cords resembling liver. Much of the tumor was composed of smaller cells of varying shape with more basophilic cytoplasm and no definite arrangement. In places transitions of cells resembling those of liver cords to less differentiated tumor cells were seen. The stroma was generally scant, although moderate amounts were present in some areas. Portions of the tumor had a dense fibrous capsule and compressed the surrounding liver, while other portions were nonencapsulated and infiltrated the liver tissue.

Considerable hemorrhage and necrosis were present. The liver parenchyma showed extensive fibrosis, especially in portal areas, with marked proliferation of bile ducts and considerable lymphocytic infiltration. There was evidence of chronic passive hyperemia of the central zones.

Cross sections of the portal vein, the superior mesenteric vein and the mesenteric vein from the appendical region showed considerable narrowing and eccentricity of lumens by partially organized, degenerated and calcified material. This material was poorly delimited from the intima, although Weigert's elastic tissue stain demonstrated that it was situated internal to the internal elastic lamina, and it was interpreted as of thrombotic origin. The media of these veins showed considerable degeneration, with loss and disruption of elastic fibers, and fibrosis. Cross sections through the coronary vein of the stomach, the "egg shell–like" structure and periesophageal veins showed thrombotic material similar to that in the portal vein, but the lumens were dilated and the material was usually disposed along one portion of the circumference. Frequently there was attenuation of the vessel wall opposite such deposits. Some calcification in the media, in addition to medial changes similar to those in the other veins, was also found. No inflammatory changes were seen in any of the sections of veins.

Sections of the splenic artery showed extensive atherosclerosis with focal calcification in the media. Partially organized thrombi with focal calcification occluded a considerable portion of the lumen, which was still greatly dilated, however. Weigert's elastic tissue stain showed interruption of continuity and loss of elastic fibers. Mallory's phosphotungstic acid–hematoxylin stain and Masson's connective tissue stain showed a marked loss of muscle fibers in the media.

Other findings of the gross and the microscopic examination are included in the anatomic diagnosis, which follows:

Anatomic Diagnosis.—Carcinoma of the liver (hepatoma); cachexia; pneumonia of the upper lobe of the right lung; mucocele and involution of the appendix with periappendical adhesions; sclerosis of the portal vein and its branches; calcific thrombi in the portal system and dilated collateral veins; portal obstruction; portal cirrhosis of the liver; esophageal varices; splenomegaly (250 Gm.) with fibrosis; moderate generalized arteriosclerosis; cirsoid aneurysm and thrombosis of the splenic artery; traction diverticulum of the esophagus, 1.5 cm. in diameter.

This case showed a number of the features of the Banti complex, but the clinical signs and symptoms were mostly related to the carcinoma of the liver. The roentgenograms showed calcifications, although these were not clinically interpreted as being in the portal system. The involvement of the one mesenteric vein draining the appendical region and the association with an involuted, adherent appendix containing a mucocele suggest that there had been thrombophlebitis of appendical origin. However, although the histologic changes in the appendix were equivocal and the periappendical adhesions suggestive, the anamnesis was silent on the subject of appendicitis. It might be added that no mention was made in the anamnesis of abdominal symptoms referable to thrombosis.

CASE 2.—A white man 38 years old was admitted because of gradually increasing abdominal enlargement and weakness of one year's duration plus dyspnea and a productive cough of six weeks' duration. He had had a single attack of severe hematemesis one year before admission, but that was never repeated.

Examination revealed: marked clubbing of the fingers; edema of the ankles; ascites; a tense spleen extending to within 3.5 cm. of the umbilicus; an enlarged liver; moist rales throughout both lungs, with dulness to percussion and absence of breath sounds on the right; very malodorous breath; a temperature of 102 F.; a systolic heart murmur over the mitral area, and a blood pessure of 90 systolic and 60 diastolic. The icterus index was 6; the red blood cell count, 3,620,000, and the white cell count, 4,900, with 74 per cent polymorphonuclears. Examinations of the urine, a Wassermann test of the blood, culture of the blood and examinations of the sputum for acid-fast bacilli all gave negative results. A roentgenogram of the chest showed density in the central portion of the right lung field.

The patient became worse and died six days after admission.

Autopsy (two hours after death).—The body was that of a well developed and nourished middle-aged white man.

At a distance of about 4 cm. from the hilus of the liver the portal vein was completely filled by a firmly adherent thrombus. In its upward extension the thrombus incompletely filled the vascular lumen. As the main branches of the portal vein were followed, a yellow discoloration of the intima with large calcific plaques was seen in many places, and calcific excrescences were found here and there, each projecting into the lumen for about 5 mm.

Microscopic examination of the wall of the vein in the region of the thrombus revealed fibrosis and hyalinization. No sign of inflammation was present. The thrombus, partly canalized, contained extensive deposits of calcific material.

Other gross and microscopic observations are included in the anatomic diagnosis, which follows:

Anatomic Diagnosis.—Gangrene of the middle lobe of the right lung with a bronchopleural fistula; right pyopneumothorax (1,000 cc.); collapse of the right lung; mitral stenosis; severe chronic passive congestion of liver; calcific sclerosis of the portal system; calcific thrombi in the portal vein; portal obstruction; distention and engorgement of mesenteric and esophageal veins; splenomegaly (1,000 Gm.) with fibrosis; edema of the lower extremities; ascites (700 cc.); hydropericardium (250 cc.).

In this case there were clinical signs and symptoms of the Banti complex, although gangrene of the lung and mitral stenosis complicated the picture. The liver did not show the typical portal cirrhosis that might have been expected but showed predominantly marked chronic passive hyperemia, evidently secondary to mitral stenosis. The sclerosis of the portal system may have been due to increased portal pressure, although there was no significant sclerosis of other veins or of arteries. The thrombi in the portal vein were probably the result of stasis and changes in the vessel wall.

COMMENT

In addition to the 95 cases of chronic occlusion of the portal vein mentioned by Simonds [1] and 23 others [2] described in the literature, there may have been still other instances reported among articles on Banti's disease and splenic anemia. An accurate tabulation is therefore difficult. In a few of the cases chronic occlusion was associated with adenoma of the liver, and in a few, with cirrhosis. In a number the occlusion was associated with calcification of the thrombi, although the presence of extensive calcification involving thrombi in tributary and collateral veins, as well as in the portal vein, was uncommon. In a few cases, the origin of the occlusion was traced to the appendix. Martos [2m] reported 2 such instances and reviewed several others from the literature. Changes in the spleen, especially enlargement, were among the most constant accompanying findings. The relationship of chronic portal occlusion to the Banti complex was emphasized by Warthin [3] in 1910.

Tortuosity of the splenic artery is a not uncommon anatomic finding in the older age groups. However, in case 1 a pronounced increase in the diameter and the length of the vessel was associated with extensive degenerative changes in the wall of the vessel. The vascular condition is described most accurately by the term "cirsoid aneurysm," although it could be classified also as a diffuse aneurysm in distinction from a circumscribed aneurysm.

Baumgartner and Thomas [4] in 1924, Anderson and Gray [5] in 1929, Bertrand and Clavel [6] in 1929 and Machemer and Fuge [7] in 1939

2. (*a*) Billman, P., and Pohl, C.: Virchows Arch. f. path. Anat. **300**:277, 1937. (*b*) Brahme, L.: Acta med. Scandinav. **61**:175, 1924. (*c*) Cabot Case 21341, New England J. Med. **213**:373, 1935. (*d*) Cabot Case 25391, ibid. **221**:500, 1939. (*e*) Carere-Comes, O.: Arch. ital. di anat. e istol. pat. **8**:158, 1937. (*f*) Caroli, J.; Guerin, P., and Scalfi, L.: Arch. d. mal. de l'app. digestif **28**:1051, 1938. (*g*) Cattabeni, M.: Gazz. internaz. med.-chir. **47**:145, 1938. (*h*) Croizat, P., and Picard, M.: J. de méd. de Lyon **20**:145, 1939. (*i*) Derow, H. A.; Schlesinger, M. J., and Savitz, H. A.: Arch. Int. Med. **63**:626, 1939. (*j*) Fontana, A.: Arch. ital. di anat. e istol. path. **2**:741, 1931. (*k*) Franke, H.: Med. Klin. **35**:1601 and 1632, 1939. (*l*) Haintz, E., and Romhanyi, G.: Ztschr. f. klin. Med. **135**:66, 1938; Orvosi hetil. **82**:1208, 1938. (*m*) Martos, J.: Arch. f. klin. Chir. **185**:322, 1936. (*n*) Osler, W.: Tr. Path. Soc. Philadelphia **14**:104, 1889. (*o*) Pallette, E. C.: California & West. Med. **45**:324, 1936. (*p*) Rix, E.: Frankfurt. Ztschr. f. Path. **53**:467, 1939. (*q*) Rousselot, L. M.: J. A. M. A. **107**:1788, 1936; Surgery **8**:34, 1940. (*r*) Strajesko, N.: Presse méd. **43**:469, 1935. (*s*) Van Creveld, S., and Levy, W. A.: Am. J. Dis. Child. **39**:790, 1930.

3. Warthin, A. S.: Internat. Clin. **4**:189, 1910.

4. Baumgartner, E. A., and Thomas, W. S.: Surg., Gynec. & Obst. **39**:462, 1924.

5. Anderson, W., and Gray, J.: Brit. J. Surg. **17**:267, 1929.

6. Bertrand, P., and Clavel, C.: Lyon chir. **26**:641, 1929.

7. Machemer, W. L., and Fuge, W. W.: Arch. Surg. **39**:190, 1939.

reviewed 92 of the reported cases of aneurysm of the splenic artery. Thirty-eight cases [8] were not included. Therefore, at the time of writing (July 1, 1941) the total number seems to be 130. While this figure represents the number of cases reported (except a case the report [9] of which was not available and which is not included here), it is not an indication of the incidence of the condition, as evidenced by figures showing the occurrence of aneurysm of the splenic artery in large autopsy series. This incidence is usually given as about 0.05 per cent of the total number of autopsies. The incidence of aneurysm of the splenic artery in the higher age groups, however, is probably considerably greater than this, as indicated by 4 arteries out of 50 mentioned by Springorum [8p] in 1933. The report of Ferrari [8e] in 1938 is also significant in that he found small or minute aneurysms occurring in 14 of a series of 143 splenic arteries from persons 60 years old or over. In 3 of these the small aneurysms occurred on the main trunk of the artery, while in the remaining 11 they occurred mostly at the points of division of branches.

In 1882 Osler [8k] described a case of chronic occlusion of the portal vein with a few areas of calcification, and in the autopsy report there was brief mention of a very tortuous splenic artery with a group of small saccular aneurysms the size of large peas at the hilus. This appears to be the only report in which chronic portal occlusion and aneurysm of the splenic artery were associated.

Trevor's [10] case of multiple aneurysms of the splenic artery was associated with calcification and dilatation of the splenic, inferior mesenteric, superior mesenteric, left gastroepiploic and portal veins. No thrombosis of these veins was present, however.

---

8. (*a*) Bauer, C. P., and Apfelbach, C. W.: Am. J. Obst. & Gynec. **24**:450, 1932. (*b*) Bohler, E.: Bull. Soc. d'obst. et de gynéc. **22**:707, 1933. (*c*) Ferrari, E., Jr.: Cuore e circolaz. **22**:585, 1938. (*d*) Fretheim, B.: Norsk mag. f. lægevidensk. **99**:1230, 1938. (*e*) Günther, G. W.: Beitr. z. klin. Chir. **168**:457, 1938. (*f*) Guy, C. C.: Surgery **5**:602, 1939. (*g*) Maljatzkaja, M. I.: Beitr. z. path. Anat. u. z. allg. Path. **94**:81, 1934. (*h*) Matronola, G.: Arch. ed atti d. Soc. ital. di chir. **45**:799, 1939; Policlinico (sez. prat.) **47**:993, 1940. (*i*) Naegeli, T.: Schweiz. med. Wchnschr. **64**:652, 1934. (*j*) Nodes, J. D. S., and Hinds, F.: Tr. Obst. Soc. London **42**:305, 1900. (*k*) Osler, W.: J. Anat. & Physiol. **16**: 208, 1882. (*l*) Östling, K.: Acta obst. et gynec. Scandinav. **18**:444, 1938. (*m*) Pasternack, J. G., and Shaw, J. R.: New Orleans M. & S. J. **92**:94, 1939. (*n*) Seids, J. V., and Hauser, H.: Radiology **36**:171, 1941. (*o*) Sperling, L.: Surgery **8**:633, 1940. (*p*) Springorum, W.: Virchows Arch. f. path. Anat. **290**: 733, 1933. (*q*) Stern, R.: Klin. Wchnschr. **4**:154, 1925. (*r*) Thiersch, H.: Beitr. z. path. Anat. u. z. allg. Path. **96**:147, 1935. (*s*) Weissenborn, W.: Chirurg **8**: 883, 1936. (*t*) Wilke: Klin. Wchnschr. **5**:1899, 1926. Warthin.[3]

9. Tabanelli, M.: Arch. ital. di chir. **54**:629, 1938.

10. Trevor, R. S.: Tr. Path. Soc. London **54**:302, 1903.

Association of primary carcinoma of the liver with either chronic portal occlusion or aneurysm of the splenic artery was not found in the literature.

SUMMARY

Two cases of chronic occlusion of the portal vein by calcific thrombi are reported. One of the patients was a white man 58 years old. The occlusion was associated with aneurysm of the splenic artery and hepatoma of the liver. The portal thrombosis in this case was probably secondary to acute appendicitis. The thrombosis involved tributary and dilated collateral veins as well as the main portal stem. The other patient was a white man 38 years old. The occlusion may have resulted indirectly from mitral stenosis. Both patients had sclerosis of the portal system.

The aneurysm of the splenic artery was of the cirsoid type. The hepatoma was associated with portal cirrhosis. Only one report of chronic portal occlusion associated with aneurysm of the splenic artery was found in the literature. Association of primary carcinoma of the liver with either chronic portal occlusion or aneurysm of the splenic artery was not found.

Laboratory Methods and Technical Notes

A METHOD FOR MEASURING CLOT RETRACTION TIME

JOHN S. HIRSCHBOECK, M.D., AND WILLIAM L. COFFEY JR., M.D.,
MILWAUKEE

Tests for measuring the extent of clot retraction in the hemorrhagic diseases have been used widely for many years. None of these methods has been entirely adaptable to the study of abnormally early clot retraction. We have recently observed that a short clot retraction time can be used as an index of susceptibility to pulmonary embolism and that heparinization may greatly prolong the retraction time.[1] We advise that the following method be used which is in part a modification of Lee and White's technic for measuring the coagulation time.

DESCRIPTION OF METHOD

The equipment needed is a clean, dry syringe with needle and two dry test tubes 1 cm. in diameter, one of which serves as a control. The tubes should be of soft glass and must be free of scratch marks. In addition they must be chemically clean. With the syringe and needle, 4 cc. of venous blood is removed, and 2 cc. is placed in each of the test tubes. The tubes are closed with rubber stoppers, and the blood is shaken for a few seconds in order to create an equal amount of foam in each tube. The coagulation time is then determined by measuring the interval of time between the complete withdrawal of the blood from the vein into the syringe and the point at which the blood fails to flow when the tubes are completely inverted. The tubes are held in the same hand and are tilted every half minute in order to test for the end point. The end point of coagulation then becomes zero for the measurement of the retraction time. The tubes are placed in a rack kept at room temperature and observed for the beginning of retraction.

There are two types of retraction. Type A is characterized by a rather sudden separation of the clot from the bottom of the tube. A clear or slightly blood-tinged crescent-shaped layer of serum is interposed between the clot and the bottom of the tube. Type B, which is more commonly seen, particularly in the more normal specimens, is characterized by a more gradual separation of the clot, usually from the sides of the tube. The serum formed may contain many erythro-

From the Medical Service of Milwaukee County Hospital and the Departments of Medicine and Physiology, Marquette University School of Medicine.

1. Hirschboeck, J. S., and Coffey, W. L., Jr.: Proc. Central Soc. Clin. Research **14**:11, 1941.

cytes and it may be difficult to decide within the limits of as long as three minutes whether retraction has begun. The appearance of a drop of serum on the surface of the clot is not a reliable index of the beginning of the retraction. Transmitted light against a dark background assists one greatly in deciding when separation has begun.

The normal clot retraction time with this method is usually between twenty-five and thirty minutes. Some normal bloods may have a retraction time as short as twenty minutes; others, as long as forty-five minutes. The clot retraction time becomes shortened postoperatively, and if it is less than ten minutes the patient may be considered to be a possible candidate for pulmonary embolism.

Forensic Medicine

NEED OF FORENSIC PATHOLOGY FOR ACADEMIC SPONSORSHIP

ALAN R. MORITZ, M.D.
BOSTON

Legal medicine is ordinarily defined as the application of medical knowledge to the needs of justice. Although by definition this would appear to be a broad and scientifically heterogeneous field, the practice of legal medicine is concerned chiefly with what might be most adequately described as forensic pathology.

It is estimated that approximately 20 per cent of all deaths occurring in the registration area of the United States result from violence or occur suddenly or unexpectedly from obscure causes. The existence in all jurisdictions of the United States of constitutional or statutory provisions which require that such deaths be investigated implies that the information thereby disclosed is essential to public welfare. Despite the fact that the law recognizes that the acquisition of reliable evidence is of sufficient importance to make medicolegal investigation of certain deaths an official procedure, there is no provision in most states to insure that such investigations shall be conducted competently. In fact, the laws are such in most places as to predispose to incompetence on the part of the official investigator.

It should be obvious to all informed persons that the acquisition and the interpretation of scientific facts relating to the circumstances and causes of death require that the investigator be possessed of special medical skill and knowledge. Even casual contact with the practice of legal medicine is sufficient to disclose the fact that many of the problems are so unique as to justify recognition of legal medicine as a special field of scientific endeavor. Although many of the objectives, much of the emphasis and not a few of the methods in medicolegal practice distinguish it from all other branches of medicine, so much of it has to do with morbid anatomy and histology as to bring it in close relation to the field of general pathology.

The medicolegal postmortem examination is more than an autopsy. In many instances it is the principal or only source of reliable information on a wide variety of important matters. Whereas the ordinary medical autopsy constitutes but one part of the scientific study of the

From the Department of Legal Medicine, Harvard Medical School.

course of an illness or an injury in a given person, the medicolegal postmortem examination, more times than not, provides the only available opportunity to acquire medical or other forms of scientific evidence.

It must be so planned and conducted that all facts of potential importance to the law, even though they are of little or no interest according to ordinary medical standards, will be acquired. From the standpoint of justice it is likely to be more important to learn whether the deceased was shot from in front or from behind than to learn that the bullet passed through the heart. It may be more important to learn that the fatal shot was fired at a range greater than 12 inches (30.5 cm.) than to discover that the deceased had severe rheumatic heart disease or carcinoma of the liver. From the standpoint of the law it may be of greater value to learn that the victim of violence had a 0.4 per cent concentration of alcohol in his blood at the time of death than to have a detailed record of the number and the sites of fractures or of the exact nature of the internal injuries. The guilt or the innocence of an accused person or the liability or the nonliability of an insurer may rest principally or entirely on medical evidence relating to the probable time of death. In the interests of justice the acquisition of medical evidence bearing on the identity of the victim of murder may prove to be fully as important as the evidence which establishes the cause of death.

The basic intellectual armamentarium of the forensic pathologist is a knowledge of morbid anatomy and histology. In addition, however, he must undertake to refer materials to, and correlate the results obtained from, many other fields of medicine, as well as from the closely related natural sciences. Thus he must be responsible for bringing the resources of toxicology, chemistry, physics, immunology, bacteriology and entomology to bear on a given problem, and when all the evidence is in, he must assume most of the responsibility of synthesizing it for those responsible for the administration of justice.

The need throughout the United States for recognition and stimulation of this potentially important but badly neglected branch of medicine provides a challenge that medical schools can hardly ignore. What specifically are some of the reasons why deaths by violence and deaths from obscure causes should be investigated, apart from the satisfaction of scientific curiosity and interest?

All violent or obscure deaths should be investigated in order that murder shall not escape recognition. A considerable proportion of all homicides are either unwitnessed or are witnessed only by persons to whose interest it is to conceal the truth. It is furthermore true that in a considerable proportion of all cases of homicide there is no external evidence of violence and no good grounds for suspecting that death has resulted from unnatural causes. An exhaustive and competent medico-

legal investigation is often required before it can be determined whether or not a crime has been committed.

Even though the cause of a given death is obvious and the manner is apparently homicide, a comprehensive medicolegal investigation is always desirable in order that objective evidence pertaining to the time, the place and the circumstances of the crime and to the identity of the victim and the suspect may be obtained. It frequently happens that the testimony of witnesses and the impressions derived from an incomplete investigation are subsequently changed so that what at first appeared to be a clear case of murder is altered to such a degree that the nonavailability of reliable objective data seriously interferes with the administration of justice.

Frequently it is essential to the protection of innocent persons against charges of criminal responsibility or civil liability that a complete rather than a perfunctory medicolegal investigation be conducted. The circumstances of a death together with the superficial appearances of the body are often misleading, and it is likely that many persons have been unjustly penalized because of the nonavailability of facts which were available only through complete investigation.

Competent medicolegal investigation of violent or obscure deaths often serves to call attention to hazards to public health and welfare which might otherwise go unrecognized either by the authorities or by the surviving dependents who may be entitled to insurance. Recognition of deaths due to preventable public hazards, of deaths resulting from uncontrolled sale of dangerous foods, drugs or beverages, of deaths due to unsuspected occupational diseases or injuries and of deaths due to unsuspected communicable diseases are but a few of the contributions to public welfare that may accrue from competent medicolegal investigations in cases of obscure death.

Medical schools may be legitimately expected to assume leadership in matters pertaining to the application of medical knowledge in the interests of public welfare. It is reasonable for a community to expect its medical school to initiate and support such medical endeavors even though these are not directly concerned with the education of practitioners or the care of the indigent sick. If the medical school is to discharge its obligations fully, it must foster such social medical services as legal medicine and public health work. Communities which support medical schools from public funds should look to their schools for aid and guidance in applying medical knowledge to the improvement of public welfare. The rendition of such aid will obviously give the medical school the right to expect financial support commensurate with the service rendered.

The official practice of legal medicine should be a joint enterprise on the part of the government and the medical school wherever possible

in order to insure maintenance of the high standards which should but which so rarely do characterize this type of medical work.

Whatever feeble inclinations the medical schools in the United States may have had in the past to foster the development of legal medicine have been thwarted in most places by the existence of the coroner system. In most jurisdictions the official responsible for medicolegal examinations is elected by popular vote on the basis of political rather than professional qualifications, and except for a few localities the scientific standard of work of the coroner's office is low. It is not likely that medical schools will be able to participate with full effectiveness in the practice of legal medicine until certain remedial legislation has been obtained.

Medical schools should exert their influence in behalf of legislation which will insure:

1. That medicolegal investigations shall become the responsibility of properly qualified persons.

2. That such persons shall be given proper statutory authority for the conduct of scientific investigations.

3. That adequate financial support be given to insure the necessary matériel and supporting personnel.

Since the potential value of properly performed medicolegal work is practically unknown throughout most of this country, it is likely that medical schools must first demonstrate the value of such work in their respective communities before adequate public funds will be allocated for the support of the work and before remedial legislation will be enacted.

The first step to be taken may be to offer to the community the services of an experienced pathologist whose duty it shall be to conduct medicolegal autopsies with full regard for the need of the law for information not ordinarily the goal of the medical autopsy. This person may be employed at first by the university and later by the state or county and the university jointly. In the beginning at least, it will probably be necessary that he function as an assistant or consultant to whatever official the law designates as being responsible for medicolegal investigations. Facilities for consultation with the various experts whose assistance may be needed in medicolegal investigations should be provided for. These should represent the fields of toxicology, chemistry, immunology, bacteriology and anatomy.

In addition to the rendition of a much needed public service, the development of legal medicine in the majority of American medical schools would correct a serious defect in undergraduate medical education. At present the students of most schools learn little or nothing of the scientific matrix of applied legal medicine. Courses of legal medicine

are characteristically confined to series of lectures on medical jurisprudence and have for their purpose the imparting of advice as to how the practitioner may avoid legal difficulties arising out of his professional activities. At the present time most physicians in the United States have little knowledge and even less interest in the field of legal medicine. Until they have some of both, progress is likely to be slow. Although it is not proposed that all students be taught to be medicolegal experts, every physician should know at least something about the field and should be aware of its potential value to society and of the desirability of having such important scientific work under the direction of properly qualified persons.

So far as undergraduate teaching is concerned, instruction in legal medicine should probably be integrated with whatever teaching there is in medical jurisprudence and in social, state or cultural medicine. The teaching of medical economics and medical ethics might well be combined with the lectures on legal medicine. Although it is not likely that such a broad field can be presented by any one instructor, its various components are so closely related that they may be profitably combined in one course of lectures.

The reasons that, and the methods by which, American schools should attempt to improve the status of legal medicine have been given. The problem of how such an enterprise can be supported demands consideration. The principal financial burden incident to the expansion of legal medicine in a medical school will lie not in the cost of undergraduate teaching but rather in the cost of supporting the practice of legal medicine for the benefit of the community.

Three potential sources of support for such an activity should be considered. The first is from public funds raised for the conduct of government. Official medicolegal practice is a recognized function of government and as such should be supported in the same manner as any other governmental activity. It may be that in any given community adequate support from public funds will not become available until the value of the work has been demonstrated. In such an event it will be necessary to secure temporary support for the project either from general school funds or from some outside source.

The responsibility of the medical schools of a country for the prevailing standards of medical practice is undeniable. The high professional standards of physicians in the United States can be attributed in a large measure to the general excellence of the nation's medical schools. The low status of legal medicine throughout most of the states is an anomalous phenomenon that demands early correction.

General Reviews

THE HISTAMINE THEORY OF ANAPHYLACTIC SHOCK

WITH SPECIAL REFERENCE TO ANAPHYLAXIS IN THE RABBIT

M. ROCHA E SILVA *
SÃO PAULO, BRAZIL

The similarity between the pharmacologic properties of histamine and the properties of the so-called anaphylactic poison generated in the sensitized body following injection of the antigen led Dale (1920; 1929; 1933), Abel and Kubota, and Lewis and Grant to the assumption that during anaphylactic shock there takes place a liberation of a hypotensive, smooth muscle–contracting substance like histamine. The demonstration of the existence of this substance in the tissues of almost all animal species was undertaken by Guggenheim and Loeffler, Abel and Kubota, Popielski, Koessler and Hanke, Best, Dale, Duddley and Thorpe, Barsoum and Gaddum, Tarras-Wahlberg, Code (1937 a and b), Martin and Valenta and many others. The theory that histamine is liberated from the tissues after the combination of an antigen with its so-called anaphylactic antibody was placed on solid ground when it was demonstrated that isolated organs of sensitized guinea pigs when perfused with Tyrode solution containing the antigen liberated conspicuous quantities of a substance indistinguishable from histamine (Bartosch, Feldberg and Nagel; Daly and Schild; Ungar and Parrot; Schild). In the same way experiments on dogs showed that during anaphylactic shock the circulating blood and especially the lymph flowing from the liver contain high amounts of a substance which cannot be distinguished from histamine (Manwaring and co-workers; Dragstedt and Gebauer-Fuelnegg; Dragstedt and Mead; Code, 1939).

The histamine theory of anaphylactic shock involves the following statements: 1. Histamine reproduces in almost every animal species the complex of symptoms characteristic of the anaphylactic crisis. 2. Histamine is encountered in almost all mammalian tissues and especially in the so-called shock organs in quantities which are sufficient to explain the most marked effects produced locally in the event that the histamine is liberated. 3. There is much experimental evidence that histamine is liberated from the tissues after the intravenous injection

From the Instituto Biologico.
* Fellow of the John Simon Guggenheim Memorial Foundation.

of the antigen into prepared animals. 4. With slight discrepancies, there is a general parallelism between the sensitivity of the different animal species to histamine and anaphylactic shock (Schmidt and Stähelin).

Although the observed facts are mostly consistent with these preliminary statements, there are many discrepancies between the pharmacologic effects of injected histamine and the symptom complex of anaphylaxis. These discrepancies encountered in certain animal species are of several orders: First *there are differences in degree between the most characteristic symptoms developed after the injection of an antigen and after that of histamine.* Grove, for instance, pointed out the differences in the effects produced on isolated lungs of rabbits by histamine and antigen, the latter producing a much more conspicuous constriction of the pulmonary vessels than the former. A general explanation of this type of discrepancy is encountered in Schild's recent statement (1939) on the quantitative difference which probably exists between the liberated histamine and the histamine added to the bath containing isolated strips of smooth muscle. Histamine liberated from the tissues may act about fifteen times more powerfully than histamine added from the outside. The fact that histamine is liberated in the inner structures of the tissues might explain the quantitative differences pointed out by Grove. Under this heading, the discrepancy pointed out by Abramson and associates should also be mentioned. Using a method of reversed iontophoresis, they attempted to obtain histamine from allergic wheals of the human skin. Taking the size of the wheals as a criterion of the probable quantity of histamine present, they observed that in no instance was histamine obtainable from the allergic wheals, while it was obtained regularly iontophoretically from histamine wheals of equivalent size. Since histamine liberated from the tissues may act fifteen times as strongly as histamine introduced from the outside, the reversed iontophoresis method may not be sufficiently sensitive to detect the presence of such minute amounts of histamine.

Second, *histamine sometimes produces an effect just the opposite of the one produced by an antigen.* As a representative example of this type of discrepancy one may cite the opposite effects produced when histamine and antigen are brought in contact with the isolated uterus of the rat: histamine regularly produces a decrease of tonus and antigen the usual contraction, as shown by Kellaway. Another example might be the discrepancy between the effect produced by histamine and that produced by the antigen on the sensitized rabbit: whereas the antigen always produces a fall of the blood pressure, histamine almost always produces an evanescent rise in the carotid blood pressure. Recently this discrepancy was the object of experimental work, which will be discussed later (Rocha e Silva, 1940 b).

Third and finally, *there are discrepancies with relation to the experimental conditions under which the reaction to histamine and that to antigen are brought forth.* Sometimes under the same conditions the antigen still reacts while histamine no longer produces its usual effect. For example, Schild (1936) showed that in the isolated guinea pig uterus, the effect produced by the addition of the antigen is not annihilated by a previous poisoning of the muscle with large amounts of histamine, while this treatment annihilates completely the effect of further addition of small amounts of histamine. Recently Feldberg and Kellaway (1938) suggested the participation of a lysocithin-like substance, such as that formed after the treatment of isolated organs with snake venoms, to explain the discrepancy just cited, and more recently Kellaway and Trethewie presented evidence that a slow-reacting substance (S. R. S.) is liberated during in vitro anaphylaxis and concluded that the anaphylactic contraction of smooth muscle structures must be due in part to the liberation of this substance. The liberation from the lungs of sensitized guinea pigs of an oxytoxic substance other than histamine was shown in the experiments of Campbell and Nicoll. Thus, the cooperation of substances other than histamine in the production of the anaphylactic effects might explain some of the discrepancies cited.

The present paper was written with the aim of alining the pharmacologic action of histamine with the symptoms of the anaphylactic crisis in the rabbit. It seems desirable that similar work be done in reference to anaphylaxis in the rat and the pharmacologic effects of histamine on this animal species, since some unexplained discrepancies are frequently cited in the literature dealing with the histamine theory as applied to anaphylaxis in the rat.

HISTAMINE AND LOCAL ANAPHYLAXIS IN THE RABBIT

It is well known that repeated injections of antigen under the skin of a rabbit lead to the development of a more or less severe inflammatory reaction characterized by hyperemia, increased capillary permeability, leukocytic migration, hemorrhages and, in more severe cases, sterile abscesses and necrosis with formation of scars. This phenomenon, first reported by Arthus, was studied and described in detail by Arthus and Breton after they had made repeated injections of horse serum into the skin of the rabbit. The designation of Arthus phenomenon, proposed by Nicolle and now generally adopted, is preferable to that of hyperergic inflammation, proposed by Roessle. Roessle's designation could be accepted only from a philosophic or teleologic point of view since it has no relation to the experimental data available. The differences between the so-called normergic and the hyperergic inflammation are rather a matter of morphologic details, being due probably to the special conditions under which the noxious agent attacks the tissue elements.

Though these differences may have some clinical or diagnostic significance, they are certainly of minor importance from the standpoint of the mechanism producing the reaction.

Although discussion has been aroused about the relationship between the Arthus phenomenon and general anaphylaxis (Besredka), there is little doubt of the close parallelism between the conditions under which the Arthus phenomenon is produced and those under which the general symptoms of anaphylactic shock develop when a sensitized rabbit receives an injection of the antigen intravenously. It has been observed that the severity of the Arthus phenomenon increases with the number of subcutaneous injections. Opie (1924a) has shown a certain parallelism between the titer of the precipitin in the blood of the sensitized animal and the intensity with which the local reaction develops after the subcutaneous injection of the antigen. Using a more sensitive method for the detection of precipitins, Cannon recently showed a definite parallelism between the precipitin titer and the intensity of the Arthus reaction elicited by repeated injections of crystalline egg albumin in both rabbits and guinea pigs. On the other hand, it is a common observation that repeated injections of antigen render more and more severe the anaphylactic crisis developed after the injection of the antigen. This fact was first verified by Arthus (1909) when he recorded the drop of blood pressure in rabbits receiving an increasing number of injections of the antigen. The same phenomenon was recently studied by Grove, who established an incomplete parallelism between the titer of the precipitin in the circulating blood and the severity with which the rabbits reacted to intravenous injections of the same amount of the antigen. That the Arthus phenomenon is produced in the same way as any other anaphylactic reaction, by the interaction of the injected antigen and the antibodies existing in the tissues of the sensitized animal, became more probable through the interesting and suggestive experiments of Auer (1920). Rabbits sensitized with horse serum were given intraperitoneally a massive dose of the antigen. Thirty minutes to three hours later their ears were rubbed gently with xylene, which produced only a mild inflammation in the control animals as well as in sensitized animals which had not received the previous injection of the antigen. In the sensitized animals, however, which a short time before received the injection of horse serum, severe reactions developed, resembling closely the Arthus reaction. The most acceptable explanation for this fact seems to be that proposed by Auer himself, namely, that the injected antigen would find its way through the capillary walls rendered permeable to protein molecules in the skin treated with xylene.

On account of the severe necrosis at the sites of the Arthus phenomenon, one might be induced to suppose that the cells of the sensitized body would be individually attacked by the foreign protein when brought

in contact with it. This view was sponsored by Opie (1924 a), who postulated a cytotoxic action of the specific precipitate formed by the interaction of the antigen with the precipitins contained in the body fluids on the areas submitted to the reaction. In contrast to this view, Meyer and Loewenthal, as well as Aranson, showed in tissue culture experiments that cells isolated from sensitized guinea pigs were not injured by contact with the antigen employed for sensitization. Similarly, Rich, Lewis and Gay showed the harmlessness of the contact of the antigen with the cells of the sensitized animal when the isolated tissues were planted in a medium consisting of mixtures of the animal's serum with the protein to which the animal had been sensitized, even when abundant precipitate was formed. That the phenomenon is primarily a hemorrhagic one, starting probably after damage of the capillary endothelial cells, was shown by the beautiful experiments of Rich and Follis, who used the corneal tissues as a site of production of the Arthus reaction. The injection of antigen into the avascular tissues of the cornea of the sensitized rabbit resulted only in slight alterations of the cells and no necrosis. When they repeated the experiment, using animals whose corneas had been vascularized by injecting a drop of an irritant a week or ten days previously, the injection of the antigen resulted in an intense inflammation, with swelling of the fibers of the cornea, leukocytic migration and rupture of capillaries with hemorrhages, simulating a miniature Arthus phenomenon. These experiments point to the conclusion that the tissue injury in the Arthus reaction results from marked alterations in the nutrition of the cells produced by the vascular damage and obstruction of the tissue spaces with exudate and products of blood origin. Thus, it is not the action of a cytotoxic product formed by the combination of antigen with antibody in the tissues which per se produces the cellular alterations appearing in the Arthus phenomenon. It seems quite probable that the action of an active angiotoxic substance formed locally or liberated from the tissues, acting primarily on capillaries and small vessels, producing increased permeability and vascular ruptures with minute hemorrhages, would lead to impairment of nutrition and cellular injury.

The inflammatory nature of the processes underlying the Arthus phenomenon has been shown by the careful morphologic studies of many investigators, for example Opie (1924 b), Roessle, Gerlach, Klinge and Ewald. Gerlach, after extensive microscopic studies, pointed out the enormous swelling of the connective tissue, the leukocytic infiltration and the stasis in the veins and capillaries following the injection of the antigen under the skin of prepared rabbits. Twenty-four hours later, the swelling of the connective tissue was considerably increased, hyalinization of the connective fibers was more and more marked, the lumens

of the capillaries had disappeared and hemorrhages had occurred. At the site of inflammation necrotic cells and injured leukocytes were observed in the disappearing lumens of the vessels. In the periphery, large leukocyte infiltration, swelling of the interstitial tissue, hyperemia, and hemorrhages, as well as hyalinization of the walls of the vessels, were shown. After some days, the site of the Arthus reaction was well delimited by granulation tissue, which became cicatricial.

Ramsdell, employing the trypan blue test, showed an increase of permeability in the capillaries of the skin of sensitized rabbits at sites of injection of the antigen. A few minutes after the intravenous injection of the dye, the areas of the ears into which the antigen had been injected presented a more and more pronounced blue color. The similarity of the blue spot developing under these conditions to that which developed after the intradermal injection of histamine has been emphasized by Ramsdell in her report of these experiments with trypan blue. I repeated the experiments of Ramsdell with the same result, using the skin of the abdomen instead of that of the ear as the site of intradermal injections. Rabbits sensitized with horse serum by the Grove procedure, with high titers for the precipitin in the blood, showed beautiful blue spots at the sites of intradermal injection of diluted horse serum five to ten minutes after the injection of a 1 per cent solution of trypan blue into the ear vein. The same experiment done with normal rabbits, which received horse serum intradermally and trypan blue intravenously, gave no such result. The injection of histamine in concentrations from 1 : 5,000 to 1 : 1,000,000 led to the formation of a homogeneous colored area with a tendency to spread (Bier and Rocha e Silva). Numerous irritant and inflammation-producing substances (a thermal irritant, turpentine, pyridine, amylic and butylic alcohol, chloroform, snake and bee venoms, trypsin, peptones) produced a similar seepage of dye into the intradermal injected areas of the rabbit (Okuneff; Menkin; Rocha e Silva and Bier [1939 b, c]; Rigdon; Rocha e Silva and Dragstedt). A comparative study of capillary fragility in rabbits with inflammation produced by chemicals, killed streptococci and Staphylococcus aureus, as well as rabbits with allergic inflammation, was undertaken by Zander, who concluded that the resistance of capillaries during the Arthus phenomenon undergoes changes that are essentially the same as those produced by inflammation in normal animals. The analogy between the mechanism of the local anaphylactic reaction (Arthus phenomenon) and that of an acute inflammation produced by an irritant substance like mustard oil has been strengthened by the experiments of Riesser showing an increased amount of histamine in the sites of rabbit skin submitted to both treatments.

Hence there is a strong analogy between the Arthus phenomenon and the inflammatory reactions observed after the intradermal injection of irritants. It is also very probable that before any appreciable tissue damage of cellular or vascular origin occurs there is liberation of a substance producing increased capillary permeability coincident with the combination of the antigen with the antibody in the skin tissues of the rabbit. The question whether this substance is histamine or a closely related substance is at present a matter of speculation, since there is only more or less indirect evidence for its support. That high amounts of histamine are bound to the tissues of the rabbit skin has been shown by numerous workers (Tarras-Wahlberg, 1937; Riesser; Rocha e Silva and Bier, 1938). The only statement one might safely accept is that enough histamine is bound to each gram of skin to account for the blue spot if this histamine should be released by irritants.

Following another line of evidence, I have shown (1940 c) that the very young rabbit (200 to 500 Gm. weight) reacts only slightly to the intradermal injection of histamine as well as to the intradermal injection of an irritant substance, such as turpentine. Notwithstanding that, the amount of histamine bound to the skin tissues is sometimes very high and far more conspicuous than it usually is in the skin of old rabbits. Thus, I explain the absence of reaction of the skin of the young rabbit to turpentine not by an absence of histamine bound to the skin tissues but rather by the insensitivity of the vessels of the skin to the liberated histamine. This explanation is consistent with the observation of Friedberger and Heim on the insensitivity of the skin of the very young rabbit to several irritants like eel serum and mustard oil injected intradermally. On the other hand, Freund has shown that the Arthus reaction does not occur in young rabbits immunized with horse serum. In most of the animals, the absence of the reaction could be anticipated, because the precipitin in the serum was slight or nonexistent, but in 2 young rabbits immunized against egg albumin the precipitin was high enough to allow the development of an Arthus reaction, but even in those animals the reaction failed to develop. So far as one can tell from these data, the skin of the young rabbit is definitely less reactive to histamine, to irritant substances and to the Arthus reaction.

Undoubtedly it would be interesting to verify in very young rabbits, by experiments on passive transference of anaphylaxis, whether the Arthus phenomenon as a mild inflammatory reaction (revealed by the trypan blue test) may be reproduced by the intradermal injection of antigen. One would overcome in this way the usual difficulty of eliciting in very young animals a satisfactory production of precipitins by active immunization. Perhaps, in this way one could have additional evidence that histamine plays an important role in the earlier stages of the Arthus phenomenon.

SMOOTH MUSCLE TONE UNDER ANAPHYLACTIC SHOCK AND HISTAMINE ACTION

From the classic work of Biedl and Kraus one might admit that the cardinal point involved in the anaphylactic shock in rabbits, dogs and cats would be the sudden fall of carotid blood pressure which follows the injection of the antigen into the veins of sensitized animals. Vomiting, defecation and urination would be merely consequences of the sudden lowering of the blood pressure. The criticism by Arthus (1920) led to a less dogmatic explanation of the anaphylactic crisis, and the subsequent work dealing with the participation of histamine during the anaphylactic shock led to the assumption of a generalized participation of increased smooth muscle tonus in the production of the symptoms. An intravenous injection of histamine into rabbits produces a sudden increase in the activity of the intestinal tract. Repeated doses produce defecation (Dale and Feldberg, cited by Feldberg and Schilf).

Auer (1911) reported increased peristaltic movements of the intestinal tract during an anaphylactic shock in the rabbit. Manwaring and Marino demonstrated that but slight smooth muscle contraction of the urinary bladder occurred in rabbits during anaphylactic shock. Bally (1929 b) reported that of 9 rabbits only 1 showed increased intracystic pressure, while 11 of 23 rabbits showed marked increase of smooth muscle tone of the intestinal tract during the reaction produced by histamine when this drug was injected into rabbits and reported that only 3 from a series of 12 rabbits showed slight contraction of the smooth muscle of the urinary bladder. By recording the pressure in the intestinal tract before and after the injection of histamine he showed definite quick smooth muscle contraction in 18 of a total of 22 rabbits tested.

Under this heading the work regarding the action of histamine and the effect of anaphylaxis on the circulatory apparatus of rabbits must be considered. The primitive conception of Mautner and of Mautner and Pick of the keys or strategic points in the circulatory apparatus of the different animal species received some morphologic support from the findings of Auer and Lewis, Coca, Simonds and Arey and Jaffé for the guinea pig, the rabbit and the dog. The question involved in this conception, however, is mainly one of quantitative differences, and the assumption that branches of the pulmonary artery in the rabbit are surrounded by a more considerable quantity of smooth muscle than are, for example, the hepatic veins and smaller arteries of the systemic circulation, would not exclude the participation of the last-named segments of the circulatory apparatus during the anaphylactic crisis. There is no doubt that during anaphylactic shock in the rabbit the pulmonary vessels are strongly contracted and the pressure in the right ventricle and the trunk of the pulmonary artery highly increased (Airila; Coca; Drinker and

Bronfenbrenner; Grove), but it would be highly improbable that the systemic arteries would not participate in the general reaction, on account of the presence up there of smooth muscle fibers. Some evidence available in the literature would lead to that conclusion. The direct observations of mesenteric arteries and the roentgenographic records of the whole body made by Valery-Radot and co-workers did provide evidence for a generalized arterial constriction during the anaphylactic shock in the rabbit. The microscopic observations made by Abell and Schenck, which will be discussed later, must be considered as satisfactory evidence pointing in the same direction. In view of such experimental evidence, the reason that under the usual conditions of recording arterial blood pressure the most outstanding effect observed after the injection of the antigen is a sudden fall of blood pressure must be discussed carefully.

Recently I showed that under special conditions of technic the situation may be reversed and a vasopressor action following the injection of the antigen may be recorded. Repeated constriction and release of the trunk of the pulmonary artery in the rabbit leads to a true state of shock. After the treatment the carotid blood pressure sinks to a low level. If at this moment one injects the antigen, the carotid pressure rises, sometimes very conspicuously. The explanation proposed by me was that the state of shock developed by this mechanical treatment is produced by exclusion of the tone of the small vessels and capillaries, which leaves to the antigen only the possibility of acting on the arterial system. This explanation was corroborated by the fact that after this treatment the injection of histamine into the veins produced a conspicuous rise of the carotid blood pressure and no fall. An alternative explanation was recently proposed by Feldberg (1941), who assumed that the successive release and constriction of the trunk of the pulmonary artery would produce diminished sensitivity of this vessel to the effect of both histamine and antigen.

The action of histamine on the circulatory apparatus of the rabbit was understood by Feldberg (1927) as being produced by the balance of two opposite effects. The pressor one, produced by the action of histamine on the arterial system, and the depressor one, exerted on capillaries and small vessels of the body. This concept provides the basis for understanding the varied effects produced by histamine when the drug is injected into rabbits' veins under different conditions of anesthesia. Under ether anesthesia the pressor effect is enhanced (Dale and Laidlaw; Feldberg, 1927) and overwhelms the depressor one. Under slight ethyl carbamate (urethane) (Dale and Laidlaw; Ackermann and Kutscher) or chloralose (Feldberg, 1927) anesthesia, rabbits react to histamine in the same way as cats and dogs. Feldberg interpreted these facts as indicating that ether and deep ethyl carbamate anesthesia produce a decrease in the tone of small vessels, which is very weak in the normal rabbit, whereas chloralose is an anesthetic less likely to weaken the capillary tone, leaving

a margin in which histamine might act on this segment of the circulatory apparatus. The technic devised by me of constricting and releasing the trunk of the pulmonary artery should supply a much more effective means of depressing that tone than ether anesthesia, since the pressor action of histamine is much more conspicuous after that treatment than it is under deep ether anesthesia. The perfect exclusion of the tone of smaller vessels under those conditions would explain why the pressor effect produced by the injection of the antigen into sensitized rabbits has been revealed in such a clear manner. If one accepts the alternative explanation proposed recently by Feldberg (1941), the final consequence of the diminished reactivity of the trunk of the pulmonary artery would be a more apparent effect of histamine and antigen on the systemic arteries producing the rise in blood pressure, but the general significance of the fact observed would be the same.

The balance of two histamine actions on the vessels of the rabbit was put into evidence in the experiments which Feldberg (1927) and Hosoya made on the ear of that species. After injection of histamine into the vein of one of the ears or iontophoretic application of the drug to the skin, reddening appeared, due to dilatation of capillaries and small vessels. At the same time a powerful contraction of the central artery, the lumen of which almost disappeared, took place. Lewis and Marvin, puncturing the ear of the rabbit with histamine, showed dilatation of the capillaries of the skin. In perfusion experiments on an isolated ear of the rabbit, Flatow showed marked constriction of the principal arterial trunks of the ear following injection of histamine. Ramsdell, injecting histamine into the skin of the rabbit and, a few minutes later, injecting trypan blue into the veins, showed that the drug produces dilatation and increased permeability of the capillaries at the site of injection of histamine. This effect was revealed by the beautiful blue spot which formed five to ten minutes after the injection of the trypan blue into the veins. My co-workers and I (Bier and Rocha e Silva, 1939; Rocha e Silva and Bier, 1939 b and c; Rocha e Silva and Dragstedt) have studied the mechanism of this trypan blue phenomenon in the rabbit skin. Contrary to Menkin's statement (1938; 1939), histamine in proper concentrations (1 to 8 micrograms) induces homogeneous accumulation of the dye in intradermal injected areas, showing definite dilatation of small vessels and increased permeability of capillaries. The apparent reason for the failure of Menkin's attempts to produce a uniform blue spot with histamine has been that he injected high concentrations of the drug (0.2 to 10 mg.) into relatively small areas of skin. Undoubtedly, these are unphysiologic amounts of histamine, the effects of which could not be checked by any previous experimental work. This action of histamine on capillaries and small vessels, dilating them and increasing the permea-

bility of the capillaries, would explain the fact cited by me (1940 b) that repeated injections of histamine lead to further and further decrease of the blood pressure, although the immediate effect produced by a single dose of the drug is a vasopressor one followed by a slight irreversible drop of the carotid blood pressure. After several injections of small doses of histamine the vasopressor action is overwhelmed by the vasodepressor one. This fact has been accounted for by the assumption that the action of the drug on small vessels and capillaries is more lasting than that on the arterial system. The rapid disappearance of the action of histamine on the arterial system was definitely shown by the experiments referred to in the foregoing paragraphs, done on rabbits prepared by repeated constrictions and releases of the trunk of the pulmonary artery. Although there was an immediate rise of pressure in the carotid artery following the injection of histamine, a rapid drop of the pressure to the initial value took place in one to three minutes.

The increased tone of the arteriolar system in sensitized rabbits was shown in the experiments of Friedberger and Seidenberg, who perfused isolated ears of rabbits with Ringer-Locke solution. After the addition of the antigen to the perfusing fluid, a general constrictor effect was shown. The technic devised by Friedberger and co-workers, however, did not permit conclusions concerning the state of the capillary and small vessels during the experiment. In fact, it is a general condition in these experiments of perfusion with recording of flow that a constriction of the main vessels should determine a diminution of flow even when the arteriolar and capillary system may be dilated. In this respect the experiments of Abell and Schenk are much more conclusive. They observed with the high powers of the microscope the behavior of the arterioles, capillaries and venules, using the transparent moat chamber in the rabbit ears. They compared the small vessels of a normal rabbit with those of a sensitized rabbit which had received an injection of the antigen. After repeated injections of the antigen into sensitized animals, they observed arteriolar contraction, with obliteration of the lumens, increased adherence of leukocytes to the endothelium of the blood vessels and leukocytic migration through the walls of the capillaries and venules. Particularly interesting was the fact that leukocytes become sticky, adhering to form clumps or emboli and producing in certain cases stoppage of the circulation in the small vessels. Endothelial destruction with blood extravasation was seen in the most severe reactions. But no contraction of capillaries or venules was detected during any of the observations.

That this clumping of leukocytes is of interest as explaining some of the peculiarities of the anaphylactic crisis in the rabbit and particularly the leukopenia which is a common occurrence during anaphylactic shock in almost all animal species was shown by the recent experiments done

by Dragstedt and associates in which they perfused the lungs of the normal rabbit with its own blood, kept unclotted with heparin (Dragstedt, Ramirez and Lawton; Dragstedt, Ramirez, Lawton and Youmans). After several control passages of the blood through the lungs, a mixture of optimal proportions of the antigen and the antibody was added to the perfusing fluid and the experiment continued for additional passages. Leukocyte counts were made before and after the addition of the shocking mixture. There was a marked reduction in the leukocyte counts, amounting to approximately 50 per cent of the preceding values, even immediately after the first passage of the blood containing the antigen-antibody mixture. Hence, the lungs or every organ luxuriantly provided with capillaries and small vessels may act like a filter for the leukocytes which become sticky after the injection of the shocking mixture.

These experiments were performed by Dragstedt and associates with the aim of explaining the lower histamine content encountered in the sensitized rabbit blood after intravenous injection of the antigen (Rose and Weil). The blood of the rabbit is the main source of histamine, and there is much experimental work proving that the leukocytes are the richest source of histamine in the rabbit's blood. Barsoum and Gaddum, using citrated rabbit's blood, have shown that the amount of histamine bound to the blood cells is six times that of the plasma. Anrep and Barsoum found a ratio ranging from 10:1 to 18:1 for the histamine equivalents corresponding respectively to blood cells and plasma. Code (1937 b) and Code and Ing submitted the question of the source of histamine in the rabbit's blood to extensive experimental work and concluded that in the rabbit 70 to 100 per cent of the total histamine extractable from the blood was first contained in the white cell layer of the centrifuged unclotted blood. Thus, the diminution of the circulating leukocytes shown in the experiments of Dragstedt and co-workers just cited would lead to a decrease in the histamine content of the rabbit's blood. Likewise, the leukopenia that follows the injection of the antigen into the veins of the sensitized rabbit must lead to a decrease of the total histamine extractable from the circulating blood. This decrease shown in the experiments of Rose and Weil was also shown by Dragstedt and co-workers in the experiments in which they perfused the lungs of rabbits with blood containing a mixture of antigen and antibody. The experiments of Katz, however, introduced a new point of view to an understanding of the relationship between histamine and anaphylaxis in the rabbit. Rabbits sensitized to egg albumin were bled by cardiac puncture and the blood heparinized and submitted to in vitro contact with the antigen. In all instances the plasma of the blood which had undergone this in vitro shock showed a marked increase in the amount of histamine, indicating an ability of histamine to diffuse from the blood cells to the

surrounding plasma as a consequence of contact with the antigen. More recently, Dragstedt, Ramirez and Lawton confirmed the findings of Katz and calculated from in vitro experiments that the amount of histamine which could be liberated in vivo from the rabbit's blood cells would be about 0.1 to 0.3 mg. per kilogram. On the other hand, they have shown that some histamine is liberated from the sensitized rabbit's lung after perfusion with the antigen.

That sometimes the histamine of the rabbit's plasma slightly increases during anaphylactic shock was shown by Rose and Weil, indicating that a part of the liberated histamine diffuses into the plasma and then circulates in the body. It is, however, more interesting to follow the destiny of the histamine which probably is liberated from the leukocytes clumped inside the capillaries and small vessels all over the body. Undoubtedly, the histamine set free in these circumstances must act directly on the tissue cells, this condition rendering highly effective even minimal amounts of histamine liberated from the leukocytes. Hence, no appreciable change in the classic concept that the most outstanding feature of anaphylactic shock takes place near the tissue structures is so far necessary.

In this way, the contrast between the action of histamine injected into the ear vein and the symptoms of anaphylactic shock in the rabbit is in every respect less significant, since the path followed by histamine injected into the vein:

Afferent vein → pulmonary vessels (—) → arterial system (+) →
→ capillaries (—) → veins (—)

is essentially different from the one followed by histamine liberated near the cells and capillaries of the body:

Capillaries (—) → venous system (—) → pulmonary vessels (—) →
→ arterial system (+).

Each of these segments of the circulatory apparatus behaves in a very different way in relation to the regulation of the arterial blood pressure, the *minus* indicating the segments which react to histamine by producing a depressor effect and the *plus* the segments which contribute to a rise of blood pressure.

PHYSIOLOGIC CHANGES OF THE CARDIAC MUSCLE

The conception of the heart as the shock organ in the rabbit led several workers to look for some striking abnormality in the function of that organ, not only in experiments in which the isolated heart was perfused with the Ringer-Locke solution but also in the in vivo experiments in which the physiologic changes produced in the cardiac muscle by the injection of the antigen into sensitized animals were recorded. In

these experiments the usual myographic records made by Langendorff's technic, as well as electrocardiographic records, were used. Some abnormalities, to be referred to again later, were recorded, but the idea that the main changes occurring in anaphylactic shock in the rabbit were consequences of damage to the heart muscle did not receive adequate support. The changes occurring during anaphylactic shock were of minor significance and could by no means be made to harmonize with the theory of heart death developed by Auer (1911).

Baker showed depression of activity of the auricles of sensitized rabbits following exposure to minute amounts of antigen. Went and Lissàk, from experiments on isolated hearts of guinea pigs, reported results which are suggestive of a liberation of choline during anaphylactic shock. They showed a definite depression of the heart following the injection of the antigen into the perfusing fluid. Wilcox and Andrus, experimenting on isolated hearts of sensitized guinea pigs, compared the effects produced by the introduction of the antigen with those produced by the administration of histamine and concluded that anaphylaxis and the reaction to histamine are qualitatively similar. This conclusion was undoubtedly opposed to that of Went and Lissàk.

Electrocardiograms of intact animals submitted to the anaphylactic reaction have shown some contradictory results. Auer and Robinson showed disturbances consisting of heart block and changes suggesting ventricular damage in 22 of 24 rabbits submitted to anaphylactic shock. Koenigsfeld and Oppenheimer studied the electrocardiographic changes produced in guinea pigs by anaphylactic shock and reported that during a mild anaphylactic crisis no change could be recorded. In the earlier stages of severe anaphylactic shock leading to death, profound changes in the conduction, leading to heart block, might be detected. These changes, however, were not specific for anaphylactic shock; they might be observed during the most varied disturbances and were probably due to impairment of the oxygen supply of the myocardium during the shock. Criep was of the same opinion. Wilcox and Andrus recorded the electrocardiographic changes presented by isolated hearts of guinea pigs and rabbits sensitized to horse serum when the serum was injected into the perfusing cannula. In both animal species the electrocardiographic abnormalities corresponded strikingly to the diminution of the coronary flow that is a consequence of the injection of the antigen. Thus, no change could be detected that was specific to the anaphylactic reaction; the changes were similar to those recorded after mechanical constriction of the coronary arteries. The experiments done with the isolated heart of the rabbit submitted to the anaphylactic reaction also have been somewhat contradictory. Gley and Pachon, using the Langendorff technic, assayed the action of certain toxic serums and failed to demonstrate any physiologic disturbances produced by the antigen in isolated

hearts of sensitized rabbits. Cesaris-Demel (1910), taking myographic records of the isolated heart of a rabbit, showed diminution in the amplitude of the beat and increase in the tonus of the heart, but by no means stoppage of the muscle. Leyton, Leyton and Sowton perfused isolated hearts of rabbits sensitized to horse serum and observed that even 20 per cent of horse serum could be added to the Ringer-Locke solution without producing trouble of importance. The mixture of horse serum with sensitized rabbit's blood, however, produced depression of the heart beat and heart block leading to irreversible stoppage of the muscle. We have made numerous records of the behavior of isolated hearts of rabbits sensitized to horse serum while subjecting them to the in vitro action of the antigen (unpublished results). At the same time the effects of minimal amounts of histamine on the same hearts were assayed. We have used the Langendorff technic and concomitantly registered the coronary flow with the syphon recorder described by Gunn. The horse serum employed as antigen did not contain histamine, as was shown on guinea pig intestine, and did not produce any effect on the normal rabbit heart. The results obtained were somewhat irregular. Sometimes the injection of the antigen had no consequences at all, but in a few experiments it was followed by a marked increase in the amplitude of the heart beat and a slight increase in the rate. A diminution of the flow through the coronary vessels was recorded. But we never observed any depression of the rate or of the amplitude of the beat even when a cloudy suspension of specific precipitate (sensitized rabbit's serum and horse serum) was injected into the cannula. Thus, our experiments did not indicate any liberation of choline as suggested by Went and Lissàk, nor did they provide any evidence confirmatory of the experimental observations of Cesaris-Demel and of Leyton, Leyton and Sowton. The slight alterations detected in our experiments were absolutely in harmony with the possibility of a liberation of minute amounts of histamine from the sensitized heart cells that were brought into contact with the antigen, since the action of small amounts of histamine, as noted by numerous workers, consists almost always in acceleration of the rhythm, increase of the amplitude of the heart and constriction of the coronary bed (Schenck; Gunn).

Hence, the similarities between the pharmacologic effects produced by histamine and those produced by the antigen even on the isolated heart of the sensitized rabbit are striking enough to be considered and are by no means contradictory. Though the possibility of a liberation of large amounts of histamine would explain the slowing and stoppage of the heart, as was noted by Cesaris-Demel and Leyton, Leyton and Sowton, as well as the heart block following the injection of the antigen, these observations must be submitted to further verification, owing to the contradictory results obtained as described in the foregoing paragraph. In our experiments we never obtained the slightest evidence

of such a considerable effect, even in rabbits submitted to a prolonged course of sensitization by a method which raised the precipitin titer in their blood to a high level.

The experiments done with the isolated heart acquire considerable interest in a discussion of the fall of blood pressure in the intact animal after the injection of antigen. The idea that the heart is the shock organ in the rabbit was developed in the theory of heart death proposed by Auer (1911), who made a direct observation of the heart during an anaphylactic shock affecting the entire animal: "About thirty minutes after the injection of the antigen, the heart suddenly slowed considerably, the right auricle apparently did not contract at all and the right ventricle filled up strongly and stopped. During the cardiac stoppage, the animal made a few convulsive movements and the heart began to beat again." Auer postulated the occurrence of a sort of cardiac rigor which could not be counteracted by intense faradic stimulation of the muscle. As stated in the foregoing paragraph, this stoppage of the heart could not be demonstrated in experiments on the isolated heart. If the cause of this heart death resided in the muscle itself, it should have been demonstrable with that preparation. On the other hand, it is well known that the subsequent work of Airila, Coca, Drinker and Bronfenbrenner and Grove did not confirm the views of Auer but attributed the stoppage of the heart to obstruction of the branches of the pulmonary artery. According to these workers, the cardiac rigor referred to by Auer was due to the inability of the heart to pump the blood through the contracted branches of the pulmonary artery. In several experiments with sensitized rabbits which received the injection of the antigen, there were noted (Rocha e Silva, 1940 b), especially when both vagus nerves were intact, slowing and stoppage of the heart approaching a true heart death. If at this moment both vagus nerves were cut, the heart suddenly recovered, and the systemic blood pressure rose to a level sometimes above the initial one. After the injection of the serum and the fall of the blood pressure, five or ten minutes were allowed to elapse, and then both vagus nerves were cut. As a rule, immediately afterward the carotid blood pressure suddenly rose, attaining a high level. From these experiments the conclusion was drawn that some central influence must play a role through the efferent paths of the vagus nerves in the production of the phenomena occurring during the anaphylactic shock in the rabbit. It must be granted that the vagal tone, and by no means an inherent condition of the heart muscle of the rabbit as described by Auer (1911), must play a dominant part in the apparent heart death of rabbits subjected to anaphylactic shock. It is certain, however, that vagal tone alone does not account for the fundamental phenomena of the changes in the circulation during an anaphylactic crisis of the rabbit, since cutting of both vagus nerves before or during the severe, fatal shock does not prevent death.

ANIMAL POISONS AND ANAPHYLACTIC SHOCK

It is a common observation that in almost all animal species anaphylactic shock presents features of an acute poisoning resembling that produced by some snake and bee venoms and characterized by generalized constriction of the smooth musculature. Particularly in the rabbit, the only way to duplicate the complex of symptoms which characterizes the anaphylactic crisis is to inject minute amounts of animal poisons and appropriate amounts of peptone, which produce micturition, defecation, fall of pressure in the carotid artery and concomitantly rise of pressure in the pulmonary artery trunk (Kellaway and Le Mesurier) strikingly similar to what occurs during anaphylactic shock. A comparative study of anaphylactic shock and the shock developed by the injection of snake venoms was first undertaken by Arthus (1920), especially with respect to the rabbit. More recently, the similarity between some of the symptoms produced by the injection of snake venoms and those produced by anaphylaxis and histamine has been the subject of much experimental work. Also the similarity of the in vitro reactions produced by both animal poisons and anaphylaxis on the smooth muscle structures was extensively studied. Kellaway (1929) described the action of Australian snake venoms on isolated uteri of virgin guinea pigs and drew attention to the resemblance between this effect and the anaphylactic contraction of isolated smooth muscle structures as first shown by Schultz in 1912 and by Dale in 1913, and suggested that the action might not be direct but indirect, by the liberation of a substance like histamine. Essex and Markowitz called attention to the close similarity between the effects produced by the venom of the rattlesnake (Crotalus horridus) and those produced by histamine and the injection of the antigen into sensitized animals. Also on surviving organs, such as the uterus of the virgin guinea pig or the virgin rat and the isolated intestine of the guinea pig, the similarity of action was striking. Kellaway and Le Mesurier studied the action of Australian snake venoms on rabbits and emphasized once more the analogy between the action of these venoms and the pharmacologic action of histamine. The vessels of the isolated ear of the rabbit responded by contraction not only to histamine but also to the snake venoms. This similarity led Kellaway and Le Mesurier to test the possibility of a liberation of histamine by the action of the venoms on living structures. The attempts they made to show liberation of histamine from perfused organs failed probably through some imperfection of the technic. Lately, Feldberg and Kellaway (1937, 1938) were more successful, showing definite liberation of histamine after perfusion of isolated lungs of several animal species with snake and bee venoms. At the same time the analogy between the pharmacologic action of these venoms and the action of injected histamine was rendered closer. Confirmatory

findings of the liberation of histamine by snake venoms were put forward as regards intact dogs by Dragstedt, Mead and Eyer.

More recently Feldberg and Kellaway (1938) and Feldberg, Holden and Kellaway extended their first results further and showed that the symptoms of snake venom poisoning might be explained not only by the prompt liberation of histamine by the venom itself but also by the formation of substances of unknown nature, generated in the body under the action of the venom on cellular components. After contact of the venom with the organs, extracts of these organs acquired pharmacologic and pharmacodynamic properties that were not present in like extracts of normal organs. For example, extracts of envenomed liver have the ability to contract guinea pig uterus and intestine, to liberate histamine from the tissues and to hemolyze red blood cells suspended in saline solution. Among the substances generated in the body by the action of the venom is lysocithin, formed by the action of the lecithinase of the venom on the lecithin of the tissues. Another substance of unknown nature, capable of producing a "slow contraction" of the guinea pig intestine, is similarly generated in the body when the venoms are brought in contact with the living structures. Hence, not only venom itself but also such substances as lysocithin and others of unknown nature contained in the extracts of "envenomed organs" might contribute synergically to produce the histamine-like effects produced by the animal poisons. Thus, the similarity between the symptoms produced by snake venoms and those of anaphylactic shock is not a mere coincidence but is the consequence of an analogy of mechanisms of action, and the study of either of these two classes of phenomena must throw light on the other. This analogy permits an understanding of the anaphylactic crisis as an acute poisoning—anaphylactic poisoning—produced by contact of the antigen with the cellular structures, and, if one considers the chronologic development of the study of anaphylaxis and that of the study of animal poisons, one must be impressed by the fact that the picture of the so-called anaphylactic poisoning has furnished the model for an accurate study of the pharmacologic effects of snake and bee venoms.

Recently I described the most important effects produced by trypsin on mammalian smooth muscles, as well as the histamine-liberating capacity of that ferment, in experiments of perfusion of guinea pig lungs (1939). I extended this study by assaying the action of crystalline trypsin on the circulatory apparatus of rabbits, cats and dogs (1940 d). It was shown that there is a close similarity between the effects produced by trypsin and the symptoms developed during the anaphylactic shock. On the other hand, the fact that minute amounts of the crystalline ferment injected into the veins of cats, rabbits and dogs produced collapse and death put trypsin in close relationship with the animal venoms, e. g., snake and bee venom. The phenomenon of desensitization to trypsin suggested that the action of the proteolytic ferment is an indirect one, by

the intermediation of an active substance set free by that action. The histamine-liberating capacity of trypsin observed in experimental perfusion of lungs furnished the basis for one explanation of its stimulating action on smooth muscles as well as of its circulatory effects in cats, rabbits and dogs. As a matter of fact, I observed that the assayed muscles could be ranged in relation to their sensitivity to trypsin in a scale very similar to that obtained by ranging them in relation to their sensitivity to histamine. Ramirez, Lawton and Dragstedt reported experiments confirmatory of our findings on the perfused guinea pig lung and definitely showed that in the intact dog the injection of trypsin is followed by an increase of the histamine content of the blood plasma. The difference between the histamine content of the liver before and after the injection of trypsin was considerable, indicating a liberation of 6 to 10 mg. of histamine base for the whole organ. These findings are in accordance with the fact I verified that the injection of trypsin into a branch of the portal vein of the dog is followed by a much more conspicuous and lasting fall of blood pressure than the injection of the same dose into the femoral vein. More recently Dragstedt and I extended further the analogy between the effects of injected trypsin and the symptoms of anaphylactic shock (Dragstedt and Rocha e Silva; Rocha e Silva and Dragstedt). The intravenous injection of 10 to 12 mg. of trypsin per kilogram into a normal, unanesthetized dog leads to a clinical picture closely similar to the one described by Richet, namely, vomiting, laborious respiration, micturition, defecation and, after thirty to sixty minutes, bloody diarrhea. The same release of heparin which follows the injection of an antigen accompanies the injection of trypsin in all animal species studied. In rabbits, the intravenous injection of trypsin produces leukopenia and a considerable drop in the total histamine content of the blood, such as was shown by Rose and Weil to occur during anaphylactic shock. In vitro contact of trypsin with heparinized rabbit blood cells leads to a shifting of histamine from the cells to the plasma like that shown to occur when a sensitized rabbit's blood is put into contact with the antigen (Katz, Dragstedt, Ramirez and Lawton).

In view of its ability to produce contraction of smooth muscle, collapse and death in dogs, cats and rabbits, trypsin may be ranged among the animal poisons which have the same capacity to liberate histamine from living tissues. The analogy between the action of these venoms and trypsin and the action of the antigen on the sensitized animal is extended even on the virgin uterus of the rat, which, on the other hand, behaves toward histamine in a very discrepant way. All the substances referred to in the foregoing discussion, including antigen in its action on the sensitized organ, produce contraction of the virgin uterus of the rat (Kellaway, 1929 and 1930; Richter; Rocha e Silva, 1940 d), whereas histamine produces definite relaxation of this smooth muscle structure. It is interesting to note that crossed desensitization

does not occur with snake venoms and antigen (Kellaway, 1929) or with trypsin and antigen (Rocha e Silva, 1941). These negative results suggest that these substances do not attack identical sites in the living structures, and it might be necessary to introduce the idea of a difference in the place of liberation of the histamine previously bound to the living tissues, the histamine liberated by the combination of antigen with antibody being probably at a site that is not attained by substances like animal poisons and trypsin.

BIBLIOGRAPHY

Abel, J. J., and Kubota, S.: J. Pharm. **13**:243, 1919.

Abell, R. G., and Schenck, H. P.: J. Immunol. **34**:195, 1938.

Abramson, H. A., and Alley, A.: Arch. Phys. Therapy **7**:327, 1937.

——Engel, M.; Lubkin, V., and Ochs, I.: Proc. Soc. Exper. Biol. & Med. **38**:65, 1938.

Ackermann, D., and Kutscher, F.: Ztschr. f. Biol. **51**:360, 1910.

Airila, Y.: Skandinav. Arch. f. Physiol. **31**:388, 1914.

Anrep, G. V., and Barsoum, G. S.: J. Physiol. **85**:36P, 1935.

Aranson, J. D.: J. Immunol. **25**:1, 1933.

Arthus, M.: Compt. rend. Soc. de biol. **55**:817, 1903; Arch. internat. de physiol. **7**:471, 1909; De l'anaphylaxie à l'immunité, Paris, Masson & Cie, 1920.

——and Breton, M.: Compt. rend. Soc. de biol. **55**:1478, 1903.

Auer, J.: J. Exper. Med. **14**:476, 1911; **32**:427, 1920.

——and Lewis, P. A.: ibid. **12**:151, 1910.

——and Robinson, G. C.: ibid. **18**:450, 1913.

Baker, B. M., cited by Wilcox and Andrus.

Bally, L. H.: (*a*) J. Immunol. **17**:191, 1929; (*b*) **17**:223, 1929.

Barsoum, G. S., and Gaddum, J. H.: J. Physiol. **85**:1, 1935.

Bartosch, R.; Feldberg, W., and Nagel, E.: Arch. f. d. ges. Physiol. **230**:129, 1932; **231**:616, 1933.

Besredka, A.: Presse méd. **45**:1712, 1937.

Best, C. H.; Dale, H. H.; Dudley, H. W., and Thorpe, W. V.: J. Physiol. **62**:397, 1927.

Biedl, A., and Kraus, R.: Wien. klin. Wchnschr. **22**:365, 1909.

Bier, O., and Rocha e Silva, M.: Compt. rend. Soc. de biol. **129**:769, 1938; Virchows Arch. f. path. Anat. **303**:325, 1939.

Campbell, D. H., and Nicoll, P. A.: J. Immunol. **39**:103, 1940.

Cannon, P.: J. Immunol. **40**:127, 1941.

Coca, A.: J. Immunol. **4**:219, 1919.

Code, C. F.: (*a*) J. Physiol. **89**:257, 1937; (*b*) **90**:349, 1937; (*c*) Am. J. Physiol. **127**:78, 1939.

——and Ing, H. R.: J. Physiol. **90**:501, 1937.

Criep, L. H.: Arch. Int. Med. **48**:1098, 1931.

Dale, H. H.: Proc. Roy. Soc., London, s.B **91**:126, 1920; Lancet **1**:1233, 1929; Bull. Johns Hopkins Hosp. **53**:297, 1933.

——and Feldberg, W., cited by Feldberg and Schilf.

——and Laidlaw, P. P.: J. Physiol. **41**:318, 1911.

Daly, I. B., and Schild, H. O.: J. Physiol. **83**:3P, 1935.

Dragstedt, C. A., and Gebauer-Fuelnegg, E.: Am. J. Physiol. **102**:505, 1932.

——and Mead, F. B.: ibid. **30**:319, 1936.

——Mead, F. B., and Eyer, S. W.: Proc. Soc. Exper. Biol. & Med. **37**:709, 1938.
——Ramirez de Arellano, M., and Lawton, A. H.: Science **91**:617, 1940.
——Ramirez de Arellano, M.; Lawton, A. H., and Youmans, G. P.: J. Immunol. **39**:537, 1940.
——and Rocha e Silva, M.: Proc. Soc. Exper. Biol. & Med. **47**:420, 1941.
Drinker, C. K., and Bronfenbrenner, J. J.: J. Immunol. **9**:387, 1924.
Einis, W.: Biochem. Ztschr. **52**:96, 1913.
Essex, H. E., and Markowitz, J.: Am. J. Physiol. **92**:317, 329 and 698, 1930.
Ewald, W.: Beitr. z. path. Anat. u. z. allg. Path. **83**:681, 1929.
Feldberg, W.: J. Physiol. **63**:211, 1927; in Luck, J. M., and Hall, V. E.: Annual Review of Physiology, Stanford University, Calif., Annual Reviews, Inc., 1941, vol. 3, p. 671.
——and Kellaway, C. H.: Australian J. Exper. Biol. & M. Sc. **15**:81, 159, 441 and 461, 1937; J. Physiol. **94**:187, 1938.
——and Keogh, E. V.: ibid. **90**:280, 1937.
——and Schilf, E.: Histamin, Berlin, Julius Springer, 1930.
——Holden, H. F., and Kellaway, C. H.: ibid. **94**:232, 1938.
Flatow, E.: Klin. Wchnschr. **8**:569, 1929.
Freund, J.: J. Immunol. **18**:315, 1930.
Friedberger, E., and Heim, F.: Deutsche med. Wchnschr. **55**:132, 1929.
——and Seidenberg, S.: Ztschr. f. Immunitätsforsch. u. exper. Therap. **51**:276, 1927.
Gerlach: Virchows Arch. f. path. Anat. **247**:294, 1923.
Gley, E., and Pachon, V.: Compt. rend. Acad. d. sc. **149**:813, 1909.
Grove, E.: J. Immunol. **23**:101, 125 and 147, 1932.
Guggenheim, M.: Biochem. Ztschr. **51**:369, 1913.
——and Loeffler, W.: ibid. **76**:325, 1916.
Gunn, J. A.: J. Pharmacol. & Exper. Therap. **29**:325, 1926.
Hosoya, cited by Feldberg and Schilf.
Jaffé, cited by Mautner.
Katz, G.: Science **91**:221, 1940.
Kellaway, C. H.: Brit. J. Exper. Path. **10**:281, 1929; **11**:72, 1930; Bull. Johns Hopkins Hosp. **60**:1, 18 and 159, 1937.
——and Le Mesurier, D. H.: Australian J. Exper. Biol. & M. Sc. **14**:57, 1936.
——and Trethewie, E. R.: Quart. J. Exper. Physiol. **30**:121, 1940.
Klinge, F.: Klin. Wchnschr. **48**:2267, 1927; Beitr. z. path. Anat. u. z. allg. Path. **83**:185, 1929.
Koenigsfeld, H., and Oppenheimer, E.: Klin. Wchnschr. **1**:849, 1922.
Koessler, K. K., and Hanke, M. T.: J. Biol. Chem. **39**:497, 1919; **59**:879, 1924.
Lewis, T., and Marvin, H. M.: J. Physiol. **62**:19P, 1927.
——and Grant, R. T.: Heart **13**:219, 1926.
Leyton, A. S.; Leyton, H. G., and Sowton, S. C. M.: J. Physiol. **50**:265, 1916.
Manwaring, W. H.; Hosepian, V. M.; Enright, J. R., and Porter, D. F.: J. Immunol. **10**:567, 1925.
——and Marino, H. D.: ibid. **13**:69, 1925.
Martin, J., and Valenta, A.: Arch. f. exper. Path. u. Pharmakol. **193**:305, 1939.
Mautner, H.: Wien. Arch. f. inn. Med. **7**:251, 1924.
——and Pick, E. P.: München. med. Wchnschr. **62**:1141, 1915.
Menkin, V.: J. Exper. Med. **67**:129 and 153, 1938; Proc. Soc. Exper. Biol. & Med. **40**:103, 1939; Dynamics of Inflammation, New York, The Macmillan Company, 1940.

Meyer, K., and Loewenthal, H.: Ztschr. f. Immunitätsforsch. u. exper. Therap. **54**: 420, 1927.
Nicolle: Ann. Inst. Pasteur **21**:128, 1907.
Nordmann, M.: Physiol. Rev. **11**:41, 1931.
Okuneff, N.: Arch. f. d. ges. Physiol. **204**:261, 1924.
Opie, E.: (*a*) J. Immunol. **9**:231, 255 and 259, 1924; (*b*) J. Exper. Med. **39**:659, 1924.
Popielski, L.: Arch. f. d. ges. Physiol. **178**:214, 237, 1920.
Ramirez de Arellano, M.; Lawton, A. H., and Dragstedt, C. H.: Proc. Soc. Exper. Biol. & Med. **43**:360, 1940.
Ramsdell, S. G.: J. Immunol. **15**:305, 1928.
Rich, A. R., and Follis, R. H.: Bull. Johns Hopkins Hosp. **66**:106, 1940.
——Lewis, M. R., and Gay, L. N., cited by Rich and Follis.
Richter, R.: Arch. f. exper. Path. u. Pharmakol. **189**:172, 1938.
Riesser, O.: Arch. f. exper. Path. u. Pharmakol. **187**:1, 1937.
Rigdon, R. H.: Proc. Soc. Exper. Biol. & Med. **42**:43, 1939.
Rocha e Silva, M.: (*a*) Compt. rend. Soc. de biol. **130**:181, 184 and 186, 1939; (*b*) J. Immunol. **38**:333, 1940; (*c*) Nature, London **145**:591, 1940; (*d*) Arch. f. exper. Path. u. Pharmakol. **194**:335, 1940; (*e*) Proc. Soc. Exper. Biol. & Med. **45**:586, 1940; (*f*) J. Immunol. **40**:399, 1941.
——and Bier, O.: (*a*) Compt. rend. Soc. de biol. **129**:1140, 1938; (*b*) Virchows Arch. f. path. Anat. **303**:337, 1939; (*c*) Arq. Inst. biol., São Paulo **10**:219, 1939.
——and Dragstedt, C. H.: Proc. Soc. Exper. Biol. & Med. **48**:152, 1941.
Roessle, cited by Nordmann.
Rose, B., and Weil, P.: Proc. Soc. Exper. Biol. & Med. **42**:494, 1939.
Schenck, P.: Arch. f. exper. Path. u. Pharmakol. **92**:34, 1932.
Schild, H. O.: J. Physiol. **86**:51P, 1936; **95**:393, 1939.
Schmidt, G. W., and Stähelin, A.: Ztschr. f. Immunitätsforsch. u. exper. Therap. **60**:222, 1929.
Scott, W. M.: J. Path. & Bact. **15**:31, 1910.
Simonds, J. P., and Arey, L. B.: Anat. Rec. **18**:219, 1920.
Tarras-Wahlberg, B.: Skandinav. Arch. f. Physiol. (supp. 1) **73**:1, 1936; Klin. Wchnschr. **16**:958, 1937.
Ungar, G.: Les substances histaminiques, Paris, J. Hermann, 1937.
——and Parrot, J. L.: Compt. rend. Soc. de biol. **123**:636, 1936.
Vallery-Radot, P.: L'anaphylaxie humaine et expérimentale, Paris, Masson & Cie, 1937.
——Hamburger, J., and Hugo, A.: Compt. rend. Soc. de biol. **119**:376, 1935.
Went, S., and Lissåk, K.: Arch. f. exper. Path. u. Pharmakol. **179**:169, 1935.
Wilcox, H. B., and Andrus, E. C.: J. Exper. Med. **67**:169, 1938.
Zander, E.: J. Exper. Med. **66**:673, 1937.

Notes and News

Society News.—The new president of the Society of American Bacteriologists is S. A. Waksman. I. L. Baldwin was reelected secretary-treasurer, and on his resignation W. B. Sarles was elected to take his place.

Deaths.—Herbert Fox, professor of comparative pathology at the University of Pennsylvania and director of the William Pepper Laboratory of Clinical Medicine, died on February 27, in his sixty-second year.

Soma Weiss, Hersey professor of the theory and practice of physic at Harvard Medical School and physician in charge of the Peter Bent Brigham Hospital, died on January 31, at the age of 43 years.

Awards.—The Royal Society of London has awarded the Copley Medal to Sir Thomas Lewis for his cardiologic researches, the Davy Medal to Henry D. Dakin for biochemical research and a medal to E. L. Kennaway for his work on the production of cancer by synthetic substances.

The gold medal of the Radiological Society of North America has been presented to Edith H. Quimby, physicist of the Memorial Hospital for the Treatment of Cancer and Allied Diseases, New York, "in recognition of her work on dosage and filtration."

University News, Appointments, Etc.—Elise S. L'Esperance has been placed at the head of a new cancer-prevention clinic for women at the Memorial Hospital for the Treatment of Cancer and Allied Diseases, New York.

Stanhope Bayne-Jones, professor of bacteriology and director of the Jane Coffin Childs Memorial Fund for Medical Research at Yale University, has been assigned to the office of the Surgeon General of the United States Army in the division of preventive medicine and epidemiology. During the absence from Yale University of Dr. Bayne-Jones, the Childs Fund, which is mainly concerned with research in cancer, will be in the charge of Ralph G. Meader.

The Gorgas Memorial Institute of Tropical and Preventive Medicine, Inc., has elected Joseph F. Siler president, Bowman C. Crowell vice president and Merritte W. Ireland, secretary. The director of the Gorgas Memorial Laboratory in Panama is Herbert C. Clark.

Journal of Neuropathology and Experimental Pathology.—This new quarterly journal will be published under the auspices of the Association of Neuropathologists. The chief editor is George B. Hassin, Chicago, and the executive editor is Joseph H. Globus, New York.

Grants-in-Aid.—Applications to the Committee for Research in Problems of Sex, National Research Council, for financial aid during the fiscal year beginning July 1, in support of work on fundamental problems of sex and reproduction, should be received before April 1. They may be addressed to the chairman, Dr. Robert M. Yerkes, Yale School of Medicine, New Haven, Conn. Although hormonal investigations continue to command the interest and support of the committee, preference, in accordance with current policy, will ordinarily be given to proposals for the investigation of neurologic, psychobiologic and behavioral problems of sex and reproduction.

The Committee on Scientific Research of the American Medical Association invites applications for grants in support of researches on problems more or less closely connected with clinical medicine and public health. For information address the committee at 535 North Dearborn Street, Chicago.

Book Reviews

Anoxia, Its Effect on the Body. Edward J. Van Liere, Ph.D., M.D., professor of physiology and dean, West Virginia University School of Medicine. Pp. 269, with 17 figures. Price $3. Illinois: University of Chicago Press, 1942.

This is a timely and scholarly monograph on a subject of interest to members of several branches of the medical profession, especially those who have to deal with aviation medicine. In this respect it supplements, but does not supplant, those monographs which have recently appeared dealing exclusively with the medical problems of aviation. But it must not be forgotten that interest in anoxia antedates the present extensive use of the airplane and that anoxia may be produced in a variety of ways which do not involve exposure to high altitudes. The author has reviewed the literature with care, and his monograph will be found to be of service to all those who have to deal with oxygen want, no matter how produced.

Dr. Van Liere has not attempted to discuss the various diseases in which anoxia may play a part. Rather he has confined himself to a discussion of experimental methods for producing anoxia, the effects of anoxia on the various organ systems and the processes by which tolerance to anoxia (acclimatization) is produced. There is no discussion of pneumonia or anemia as such; they are mentioned incidentally as conditions which may produce anoxia. Of the twenty-two chapters, four are devoted to historical background, definitions, classification and other introductory matter. Two chapters deal with experimental methods and general considerations. All but two of the remaining sixteen chapters are devoted to the effects of anoxia on various body functions or metabolic processes; altitude sickness and acclimatization are discussed in two chapters. These subjects are carefully treated and the studies of various investigators are discussed with an appreciation made possible by the author's considerable experience in this field.

The book is remarkable for an absence of errors, either of fact or of typography. The author's analyses of experimental studies are clear and seem to avoid any misinterpretation of the views of others. Each section is concluded by a summary expressing his judgment of the present state of knowledge regarding the aspect of anoxia under consideration. The reviewer would like to have found a more liberal expression of the author's views on controversial subjects, and a greater amount of theoretic discussion, in addition to the mass of factual material presented. The author does, however, carefully emphasize the various aspects of the subject on which present information is most incomplete and calls attention to further research required in this field; therefore, his volume should prove not only an excellent source of reference material but a stimulus for further investigations.